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Der Einfluss chronischer Hypoxie entsprechend 1,000—8,000 m Höhe auf die Erythropoiese der Ratte

von

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Abstract

TRIBUKAIT B *Der Einfluss chronischer Hypoxie entsprechend 1 000—8 000 m Höhe auf die Erythropoiese der Ratte* Acta physiol. scand 1963 57 1—25 — A device is described for the maintenance of a constant low air pressure Rats were held under hypoxia equivalent to 1 000—8 000 m altitude for several months Total hemoglobin was determined according to a modified alveolar CO method Total hemoglobin at sea level was about 0 75 g/100 g body weight, increased significantly at 1 000 m and to 1 1 g at 4 000 m. A maximum of 2 ? g was at 6 000 m The limit of survival was reached at 8 000 m New levels of total hemoglobin was reached after ?0 and 40 days at 5 000 respectively 6 000 m An additional supply of iron had no effect Hemoglobin per 100 ml. blood was 14 19 and ?6 g at sea level 4 000 respectively 6 000 m Hematocrite was 45 at sea level 60 and 65 at 4 000 m and 6 000 m respectively Hemoglobin concentration parallels hematocrite values during hypoxia Blood volume increased by ?0—50 at 6 000 m but plasma volume decreased by 10—20 Hemoglobin per unit body weight decreased with increasing body weight in animals adapted to 5 000 and 6 000 m also the blood volume at 6 000 m The daily production of hemoglobin was calculated from the total hemoglobin and the assumed life span of 60 days for erythrocytes It increased to 4—5 times the normal during the first two days regardless of degree of hypoxia, but was thereafter a function of the degree of hypoxia

O Mangel wird als einer der fundamentalen Reize der Erythropoiese angesehen Das Schrifttum der umfangreichen Untersuchungen zu dieser Frage die bis auf die PAUL BERT s (1878) zuruckgehen ist u a von GRANT und ROOT (1952) HIRSJARVI (1953) NORDSTROM (1957) und MÖLLER (1960) zusammengestellt worden

Die zahlreichen Versuche naheren Aufschluss uber Art und Weise des Zusammenhangs zwischen O_2 Mangel und Erythropoiese zu gewinnen sind bislang nicht erfolgreich gewesen Die theoretische Seite dieser Fragestellung ergibt sich aus dem ausserordentlich komplexen Geschehen wie es die Dynamik von Blutbildung und Blutabbau zusammen mit dem O_2 Bedarf und der O_2 Versorgung des Gewebes darstellt Dabei sind die verschiedensten Funktionen und Systeme des Organismus beteiligt angefangen bei der Zellteilung und der Hamoglobinsynthese bis zur Regulation der Durchblutung der Gewebe

Rein praktisch ist es notwendig Grossen zu messen durch deren Hilfe mit Sicherheit ein spezifischer Effekt auf die Blutbildung erkannt werden kann Relative Blutwerte wie die Erythrozytenzahl der Hamatokrit oder die Hb konzentration einer Blutprobe sind wegen der haufig mit Hypoxie verbundenen Flussigkeitsverschiebung vom und zum Blut oft nur von beschranktem Wert und lassen zudem nicht Veranderungen der Absolutgrossen erkennen Kenntnisse der gesamten Erythrozyten oder Hb menge erscheinen deshalb aufschlussreicher liegen jedoch nur in beschranktem Umfang vor Den Untersuchungen von HURTADO MERINO und DELGADO (1945) sowie MERINO (1950) an Hohenbewohnern stehen Studien am Hund von REISSMANN (1951) und an der Ratte von FRYERS (1952) zur Seite Der fur das Verstandnis der Regulation der Erythropoiese interessante Zusammenhang zwischen verschiedenen O_2 Drucken der Einatmungsluft und der Gesamt Hb oder Erythrozytenmenge scheint jedoch nicht im Einzelnen untersucht worden zu sein

In der vorliegenden Arbeit soll die Wirkung von Hypoxie entsprechend 1 000—8 000 m Hohe auf die Erythropoiese der Ratte behandelt werden Zunachst wird ein sich selbst regulierendes einfaches Unterdrucksystem beschrieben Weiter wird die Anwendbarkeit einer Modifikation der sogenannten alveolaren CO Methode beim hohenadaptierten Tier mit der beliebig oft die Gesamthamoglobinmenge bestimmt werden kann uberpruft Damit wird nicht nur die Gesamtmenge Hb nach vollzogener Hohenadaptation gemessen sondern auch eingehend deren Bildungsverlauf untersucht In einer Versuchsserie wird festgestellt ob die normale Fe Zufuhr der erhohten Hb bildung genugt — eine nicht unwichtige Frage wenn man die Wirkung verschiedener Hypoxiegrade miteinander vergleichen will Neben diesem moglichen exogenen Faktor spielt wie sich gezeigt hat ein endogener — Alter bzw Tiergrosse — eine gewisse Rolle worauf gleichfalls eingegangen wird Relative Blutwerte sind hier nur so weit untersucht worden als es notwendig erschien die prinzipiellen Verhaltnisse darzulegen Ausfuhrlichere Daten uber die relativen Blutwerte finden sich *u a* bei SUNDSTROEM und MICHAELS (1942)

ERYTHROPOIESE DER RATTE 3

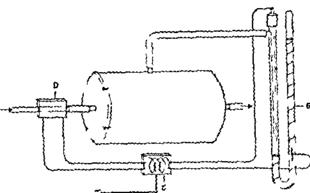


Abb 1 Halbschematische Abbildung des Unterdrucksystems Beschreibung Text

Methodik und Material

1 *Unterdrucksystem*

Es erschien am einfachsten bei den langdauernden Versuchen mit verschiedenen Graden von Sauerstoffmangel Hypoxie durch Unterdruck hervorzurufen Zu dem Zweck wurden Kerne von Autoklaven mit einem Rauminhalt von etwa 50 l mit Beleuchtung und einem Fenster versehen Die Kammern wurden durch elektrisch betriebene Pumpen belüftet deren Leistung bei 0 m etwa 70 l/min und einem Unterdruck entsprechend 8 000 m Höhe etwa 40 l/min betrug Unter keiner Versuchsbedingung reicherte sich CO oder Feuchtigkeit in den Kammern an Die Temperatur betrug 18—22 C

Ein von Schwankungen der Pumpenleistung und des äusseren Barometerdrucks unabhängiger und leicht einzustellender konstanter Unterdruck in der Kammer wurde durch ein selbstregulierendes System erreicht wie es Abb 1 halbschematisch wiedergibt Die Kammer (A) steht mit einem absolutmessenden Quecksilbermanometer (B) in Verbindung in dessen mit dem Unterdruck verbundener Schenkel durch eine Teflondichtung ein verschiebbarer spitzer Eisenstab eingeführt ist Steigt der Unterdruck und damit auch das Quecksilber in diesem Schenkel, und berühren sich Quecksilber und Eisenspitze wird über ein Relais (C) ein elektromagnetisches Ventil (D) das der Kammer vorgeschaltet ist geöffnet und der Unterdruck sinkt Bei der relativ hohen Pumpenleistung war es zweckmässig ausserdem kontinuierlich Luft über ein ständig geöffnetes verstellbares Ventil einzulassen Der Kammerdruck variiert nicht mehr als ± 2 mm Hg

Bis zu 10 kleine Tiere finden in einer Kammer Platz Eine Schleuse ist nicht vorhanden d h alle Handhabung an den Tieren und in der Kammer müssen in Meereshöhe vorgenommen werden

2 *Bestimmung von Gesamthämoglobin und Blutvolumen*

In einer vorangegangenen Arbeit ist eine Methode beschrieben worden mit der unter Anwendung von CO und einem geschlossenen Atemsystem wiederholt vom selben Tier die Gesamthämoglobinmenge bestimmt werden kann (TRIBUKAIT 1960 a) Das Gleichgewicht zwischen der Gasphase von O_2 und CO einerseits und der Sättigung von Hb mit O_2 und CO andererseits wird in einem solchen System von den Partialdrucken O_2 und CO sowie einer Gleichgewichtskonstanten K_M bestimmt Diese von Haldane für ein reines Hb-Gassystem mit M bezeichnete Konstante ist in vivo empirisch

Tab. I. Gefundene Gleichgewichtskonstanten K_M von Ratten unter verschiedenen Hypoxiebedingungen

n = Anzahl der Versuche

Unterdruck, m	n	K_M	Tage in Unterdruck
0	24	220.8 ± 3.9	–
2 000	9	207.7 ± 5.1	14
	6	207.2 ± 2.4	28
4 000	9	201.0 ± 2.5	11
5 000	8	178.3 ± 5.4	3
6 000	5	176.5 ± 6.3	2
	4	172.0 ± 2.5	3
	5	176.7 ± 8.6	4
	20	173.5 ± 2.5	30–100
7 000	10	169.2 ± 6.7	4
8 000	2	181.8	2
		168.1	

unter der Annahme eines alveolaren CO_2-Drucks von 40 mm Hg bei Normaltieren bestimmt worden. Sie hat einen Nennwert von 221.

Da in Hypoxie durch Hyperventilation der alveolare CO_2-Druck absinkt und damit der alveolare O_2-Druck entsprechend ansteigt, ist eine scheinbare Änderung dieses Wertes zu erwarten, wenn man weiterhin mit einem alveolaren CO_2-Druck von 40 mm Hg rechnet. Die Bedeutung einer Änderung des K_M-Wertes für die Bestimmung der Gesamthämoglobinmenge geht aus der folgenden Berechnung hervor: Sinkt K_M von 221 auf 200 bzw. 175 und 150, steigt bei einem hypoxischen Tier mit etwa 4 g Gesamt-Hb dieses auf etwa 4.4 bzw. 5.0 und 5.6 g.

Da keine sicheren Angaben über den alveolaren CO_2-Druck der Ratte unter Hypoxie vorliegen und da weiterhin durch eventuell Änderungen des Hb unter Hypoxie (fetales Hb) ein verändertes Bindungsverhalten für CO nicht [illegible] auszuschließen ist, wurden K_M-Faktoren unter verschiedenen Hypoxiebedingungen bestimmt (Für methodologische Einzelheiten s. TRIBUKAIT 1960 a). Alle Werte wurden unter der Annahme eines unveränderten alveolaren CO_2-Drucks von 40 mm Hg berechnet.

Bei den verwendeten Tieren handelt es sich um männliche [illegible] g schwere Wistar-Ratten. Bis zu einem Unterdruck entsprechend 6 000 m Höhe wurden die Tiere direkt auf die gewünschte Höhenstufe gebracht. Da Ratten [illegible] von Meereshöhe direkt 7 000 m Höhe ausgesetzt wurden, nicht überlebten, befanden sich die Tiere dieser Gruppe zunächst für 16 Tage in 6 000 m Höhe. [illegible] 8 000 m Höhe überlebten nur 2 Tiere von 9 trotz mehrwöchentlicher Adaptation an 2 000, 4 000 und 6 000 m.

In Tab. I sind die Ergebnisse zusammengefasst. Klar [illegible] Veränderungen [illegible] K_M sind unter allen Bedingungen festzustellen. Zwischen 0–4 000 m Höhe (pO_2 160–97 mm Hg) fällt K_M linear ab. Durch Interpolation erhält man leicht die K_M-Werte für 1 000 und 3 000 m: 214 bzw. 205. Beim Übergang von 4 000 auf 5 000 m (pO_2 97–85 mm Hg) sinkt K_M sprunghaft und ändert sich zwischen 5 000 und 8 000 m (pO_2 85–[illegible]) nur noch unbedeutend. Die K_M-Werte sind von der Dauer des Unterdruck-[illegible]

tisch unabhängig In 2 000 m besteht kein Unterschied zwischen 14 und 28 Tagen und in 6 000 m zwischen 2 und 100 Tagen. Die Werte von drei verschiedenen Versuchsreihen in 6 000 m zwischen 39 und 100 Tag sind deshalb zusammengefasst worden Die gute Reproduzierbarkeit trotz der ganz verschiedenen Zeitpunkte der Bestimmungen und des absichtlich gewählten differierenden Gewichts und Alters der Tiere bedeutet weiter dass individuelle Variationen der Tiere ohne nachweisbare Bedeutung sind

Die beschriebenen Veränderungen der K_M-Werte sind hier lediglich aus praktisch methodologischen Gründen von Interesse. Dabei ist es bedeutungslos, ob diese Abweichung von K_M nur eine scheinbare infolge eines niedrigeren CO_2-Druckes ist oder auch auf einer veränderten CO-Affinität unter Hypoxie neugebildeten Hb beruht Diese Frage ist jedoch in anderem Zusammenhang wichtig worauf in einer späteren Arbeit eingegangen wird (TRIBUKAIT 1962 d)

Es ist weiter notwendig die Bedeutung von Myoglobin und im Gewebe fixierten Hb für die verwendete CO-Methode abzuschätzen Aus den Daten von Myoglobinmenge, Totalknochenmark, Hb-konzentration des Knochenmarks sowie aus den CO—O_2-Druckverhältnissen im Gewebe und Knochenmark ist für das normale Tier der dadurch bedingte CO-Verlust auf etwa 5 % der gesamten aufgenommenen CO-Menge berechnet worden (TRIBUKAIT 1960 a) Hinsichtlich des Myoglobins weicht das höhenadaptierte Tier vom normalen kaum ab DRABKIN (1947 1948 sowie ANTHONY ACKERMAN und STROTHER (1959) fanden praktisch unveränderte Totalwerte sodass entsprechend früheren Berechnungen nicht mehr als 1—2 % der gesamten aufgenommen CO-Menge an Myoglobin gebunden werden durfte

Für nichtzirkulierendes Hb liegen die Verhältnisse beim Höhentier komplizierter Während der maximalen Hb-synthese unter Hypoxie kann die Neubildung den Normalwert um das 4—5-fache überschreiten. Eine derartige Mehrleistung kann entweder dadurch zustande kommen dass die Zellbildungs- und Teilungsprozesse beschleunigt ablaufen — diesbezüglich Untersuchungen haben bisher nicht in allen Punkten eine eindeutige Antwort gegeben machen jedoch eine gewisse Beschleunigung wahrscheinlich (ALPEN und CRANMORE 1959 a, b MOLLER 1960) Oder aber dadurch dass die Menge des zellbildenden Muttergewebes ansteigt Nach den Untersuchungen von HUDSON 1958 1960 am höhenadaptierten Meerschweinchen ist die absolute Knochenmarksmenge unverändert, der Anteil von Fettzellen im aktiven Knochenmark zugunsten erythrozytärer Zellelemente eingeschränkt, das distale Knochenmarkfettgewebe jedoch unverändert Entsprechend steigt auch die Hb-Knochenmarkskonzentration der hypoxischen Ratte etwas

Das ergibt sich aus folgenden neueren Untersuchungen Ratten die 4 Wochen lang 6 000 m Höhe ausgesetzt worden waren, wurden zunächst Cr^{51}-gezeichnete Erythrozyten injiziert Nach 10 min — eine Zeit die als genügend angesehen wurde eine gleichmässige Verteilung der Cr^{51}-gezeichneten Erythrozyten zu erreichen wurden die narkotisierten Tiere mit O-gesättigter körperwarmer NaCl Lösung bis zum Herzstillstand (20—30 Min) durchgespült. Das Hb vom Knochenmark des Femur wurde in 0,1 %iger Na_2CO_3-Lösung extrahiert und als Pyridin Hämochromogen spektrophotometrisch quantitativ bestimmt (PAUL, THEORELL und ÅKESSON 1953). Aus der spezifischen Aktivität dieses Extraktes (= zirkulierendes Hämoglobin) und der gemessenen Quantität Hb ergibt sich die im Knochenmark fixiert Hb-menge 0 019 g ± 0 000 g Hb/g Knochenmark Mittelwerte von 8 Tieren) Dieser Wert weicht signifikant von dem gefundenen Normalwert 0 015 g Hb/g Knochenmark ab (p = 0 02—0 01 s TRIBUKAIT 1960a Die gesamte Hb-menge des Knochenmarks eines 250 g schweren Tieres steigt jedoch dabei nur von etwa 0 08 g auf 0 1 g oder von rund 4 % auf 5 % der gesamten Hb-menge

Hier muss auf einen weiteren früher nicht b achteten Punkt hingewiesen werden. Im Knochenmark liegen unter Normalbedingungen eine grössere Menge nicht streng fixierter Retikulozyten — nach SAATHOFF (1950 a) und FINCH (1959) kann mit einem Knochenmark Blutverhältnis von 1 1 gerechnet werden oder 2—3 % der zirkulieren den Hb-menge entsprechend der Blutretikulozytenkonzentration Diese Retikulozytenmenge die zumindest während der ersten Zeit von Hypoxie auf etwa das 3 fache steigt (SAATHOFF 1950 a) dürfte in die mit CO bestimmte Gesamthämoglobinmenge mit eingehen gehört aber streng genommen nicht mit zum zirkulierenden Hb Andererseits ist anzunehmen dass diese Zellen beim Durchspülen der Tiere mit zum grossen Teil aus dem Knochenmark entfernt und somit nicht mit unter im Knochenmark fixiertem Hb" erfasst werden

Schliesslich ist noch in diesem Zusammenhang die Bedeutung der Milz als blut bildenes Organ unter Hypoxie zu beachten SAATHOFF (1950 a) fand mit histologischer Technik eine kräftige Erythropoiese in der Milz des hypoxischen Meerschweinchens Besonders aufschlussreich sind Untersuchungen der DNA Synthese von RA BACH COOPER und ALT (1954) bei der Ratte Während das Knochenmark die Zellneubildung etwa verdoppelt erreicht die Milz eine etwa 10-fache Neubildung

Das Blutvolumen wurde aus dem Gesamt Hb und der Hb-konzentration des Schwanz blutes × 0 75 berechnet 0 75 ist ein Umrechnungsfaktor für den Körperhämatokrit in den auch die Differenz von Hb konzentration und Hämatokrit zwischen Schwanz blut und zentralem arteriellen bzw venösen Blut eingeht Diese betrug für den H mato krit von 23 Tieren 13 6 ± 1 2 Es besteht keine statistisch zu sichernde Differenz zwischen diesen Werten und denen von Normaltieren Die Hb konzentration und der Hämatokrit wurden in der früher angegebenen Weise bestimmt (TRIBUKAIT 1960 a)

Das Plasmavolumen und das Erythrozytenvolumen ergeben sich aus dem Gesamt Hb der Hb konzentration und dem Hämatokrit entsprechend

$$\text{Tot Pl Vol} = \frac{\text{Tot Hb} \times 100}{\text{rel Hb}} \times \frac{100 - \text{Hct}}{100}$$

und

$$\text{Tot Ery Vol} = \frac{\text{Tot Hb} \times 100}{\text{r l Hb}} \times \frac{\text{Hct}}{100}$$

3 *Tiermaterial allgemeine Versuchsbedingungen*

Alle Untersuchungen wurden an männlichen Ratten eines Stammes (Wistar) aus geführt Die Grösse der Tiere variierte zwisch n 250 und 450 g innerhalb einer Ver suchsgruppe jedoch im allgemeinen wesentlich weniger als ± 30 g vom Mittelgewicht Derartige gro se Gewichtsunterschiede zwischen den e nzelnen Gruppen waren bei der teilweise mehrmonatigen Dauer der jeweiligen Versuche mit oft erhebl cher Ein wirkung auf den Zuwachs der Tiere und einer gesamten Versuchszeit von mehr als 3 Jahren nicht zu vermeiden Unabhängig davon kann jedoch die Homogenität des Tiermaterials soweit sie aus dem hämatologischen Verhalten normaler Ratten zu erkenn n ist als relativ zufriedenstellend angesehen w erden Signifikante Abweic ungen von den für diesen Ti rstamm beschriebenen Normalwerten (TRIBUKAIT 1960 b) finden sich nicht

Die Tiere erhielten ein spezielles Rattenbrot (Zusammensetzung s TRIBUKAIT 1960 b) sowie Hafer Mohrrüben und Wasser ad l bitum Zum Füttern und R inig n der Kamm rn wurden die Tiere in 2 tag gen Abständen für etwa 5 min auf Me reshöhe gebracht Verschied ne Untersuchungen an den Tieren überschritten nicht 2 Stunden

Für die Hohen Druck Beziehung wurde d Standardatmosphäre entspr chend DIN 5450 verwendet

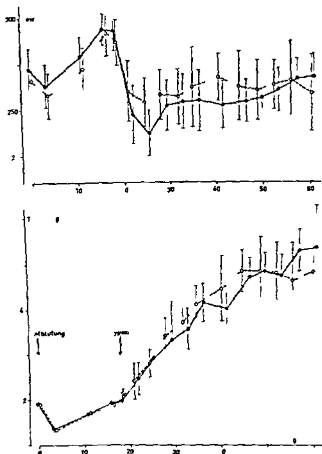


Abb Gewicht und Gesamthämoglobin von Normaltieren (offene Symbole) und Tieren mit Eisenzusatz (geschlossene Symbole) bei gesteigerter Erythropoese Mittelwert von je 7 Tieren mit Standardabweichung Blutentnahme am Tag 0 Beginn der Hypoxie entsprechend 6 000 m Höhe am Tag 18

Ergebnisse

1 *Der Einfluss erhöhter Fe Zufuhr bei gesteigerter Hämoglobinbildung*

Natürliche Voraussetzung einer optimalen Erythrozyten und Hb-bildung ist die adäquate Zufuhr der zu den Syntheseprozessen notwendigen Grundsubstanzen ASCHKENASY (1949) hat diese in einer ausführlichen Arbeit zusammengestellt (weitere Literatur s DINNING 1962)

Eisen das in relativ grossen Quantitäten vom Organismus umgesetzt wird spielt in diesem Zusammenhang eine nicht unbedeutende Rolle vor allem bei gesteigerter Erythropoiese Entsprechend tritt ein relativer Eisenmangel unter Hypoxie sehr deutlich zutage (CRISCLOLO CLARK und MEFFERD 1955 — Versuche an Ratten) Auch für den Menschen ist bei Höhenexpeditionen ein

Tab. II. Der Einfluss erhöhter Fe-Zufuhr auf die Blutwerte von Ratten bei gesteigerter Erythropoiese
a = Normaltiere b = Fe-Tiere I = vor Blutentnahme II = nach Regeneration 18 Tage
Mittelwerte von je 7 Tieren.

	Gewicht g		Tot. Hb g		Hb Konz. g %	
	a	b	a	b	a	b
I	265 ± 3.3	270 ± 4.3	1.90 ± 0.078	1.87 ± 0.051	12.53 ± 0.114	12.39 ± 0.286
II	286 ± 5.4	285 ± 3.5	2.06 ± 0.059	1.97 ± 0.074	12.73 ± 0.[illegible]28	13.01 ± 0.280
III	258 ± 8.7	267 ± 5.0	4.83 ± 0.274	5.34 ± 0.373	24.40 ± 0.336	24.[illegible]8 ± 0.709

beschleunigender Einfluss auf die Hb-bildung durch zusätzliche Eisenmedikation beschrieben worden (WINTERHALTER 1960).

Es ist vollständig ausgeschlossen, im Rahmen der vorliegenden Arbeit auch nur annäherungsweise überprüfen zu wollen, ob im einzelnen die verfutterte Tiernahrung allen diesbezüglichen Kenntnissen voll entspricht. Man muss sich hier mit der rein praktischen Feststellung begnügen, dass das Futter gemessen am allgemeinen Gesundheitszustand und Zuwachs der Tiere einer offenbar weitgehend optimalen Zusammensetzung zu entsprechen scheint. Lediglich ein einfacher Versuch in dieser Richtung wurde unternommen: bei stark erhöhter Hämatopoiese wurde geprüft, ob ein besonderer Eisenzusatz einen Effekt auf die Gesamthämoglobinmenge hat oder nicht.

Bei 2 Gruppen von je 7 Tieren gleichen Alters und Gewichts wurde eine gesteigerte Erythropoiese hervorgerufen, zunächst durch eine akute Blutung (Abnahme von etwa 1/3 des Blutvolumens durch Punktion der V. cava inf.), nach erfolgter Regeneration durch Hypoxie entsprechend 6 000 m Höhe etwa 6 Wochen lang. Eine Gruppe erhielt im Trinkwasser zusätzlich Eisen (100 mg Fe_2SO_4/500 ml H_2O — 200 mg Zitronensäure/500 ml H_2O, um einen Ausfall von Eisen zu vermeiden), die andere Gruppe nur mit Zitronensäure versetztes Wasser.

Abb. 2 zeigt das Verhalten von Gewicht und Gesamt-Hb der beiden Gruppen. Man erkennt leicht, dass eine erhöhte Eisenzufuhr weder während Anämie noch Hypoxie die Hb-bildung besonders beeinflusst. Statistisch zu sichernde Unterschiede sind nicht nachweisbar. In Übereinstimmung mit grösseren Versuchserien (TRIBUKAIT 1960 c) ist der Regenerationsprozess der dem Blutverlust am Tag 0 folgt, nach etwa 18 Tagen abgeschlossen. Der am 18. Tag einsetzende O_2-Mangel hat eine sehr kräftige Hb-neubildung zur Folge, die etwa 2 Wochen lang ziemlich unverändert anhält und schliesslich nach etwa 4 Wochen zu einer 2—3-fachen Gesamthämoglobinmenge führt. Es erscheint bemerkenswert, dass die tägliche Hb-bildung unter Hypoxie klar die unter Anämie überschreitet.

nach e n r Blutentnahme III = nach 42 Tagen Hypox e entsprechend 6 000 m Hohe.

Blutvol ml		Hct,		Hb g × 100 / H t.	
a	b	a	b	a	b
18 6 ± 0 31	18 9 ± 0 6?	39 6 ± 0 6	40 1 ± 0 60	31 6 ± 0 50	30.8 ± 0.56
?1 6 ± 0 81	20 2 ± 0 41	40 6 ± 1 00	42 0 ± 1 12	31 4 ± 0.2?	31 0 ± 0 16
?6 6 ± 1 0?	26 6 ± 0 73	74 7 ± 1.54	78 3 ± 1 41	32 ± 0 44	31 6 ± 0 64

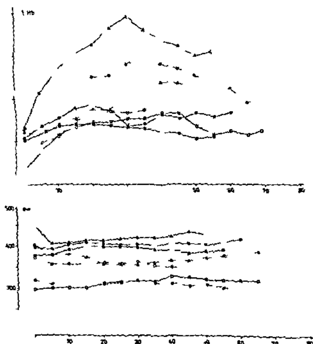


Abb 3 Gewicht und Gesamtham globin n Ratt n n Hypox entsprechend 2 000—5 000 m Hoh M tt lwert n) 5—7 T eren. Symbol m t Versu hsbe e chnung ■—■ 2 000 m (19 7 I b) ●—● 3 000 m (19 7 II a) □—□ 4 000 m (1958 I a) ○—○ 4 000 m (1957 III a) —— 4 000 m (1957 I c) ●—● 000 m (1958 I b) ▲—▲ 000 m (1957 IV) D n Pa enthese angeg benen Versu h nummern entsprechen den Be chnungen in Tab III Folgend Versu h gruppen befanden i h zuvor auf einer niedr gerer Unterdruckstuf 1957 I b 24 Tag in 1 000 m 19 7 I c 55 T ge n 000 m 19 8 I b 68 Tage in 4 000 m.

Tab III Mittelwerte von Gesamthamoglobin Korpergewicht Gesamthamoglobin/100 g Kö per hinter den Ziffern einiger Versuchsgruppen kennzeichnen gleiche verschiedenen Hohenstufen aus Standardatmosphare DIN 5450

n = Anzahl der Tiere

Versuch Nr	n	Hohe m	Barom. Druck, mm Hg	Tage in Hypoxie	Gewicht g
					Vor Hypoxie
1957 I a	7	1 000	674	24	382 ± 19 8
1957 I b	7	2 000	596	55	380 ± 20 9
1960 I	9	2 000	596	14	428 ± 7 5
1960 I	9	2 000	596	28	428 ± 7 5
1957 II a	5	3 000	526	60	398 ± 16 4
1957 I c	6	4 000	462	75	404 ± 8.2
1957 III a	5	4 000	462	40	374 ± 10 0
1958 I a	6	4 000	462	68	298 ± 19 6
1958 I b	6	5 000	405	63	320 ± 19 8
1957 IV	5	5 000	405	55	451 ± 23 8
1957 II b	5	6 000	354	30	430 ± 16 9
1958 II	6	6 000	354	40	293 ± 4 3
1958 III	8	6 000	354	40	287 ± 4 4
1958 IV	6	6 000	354	73	306 ± 12 5
1959 I	7	6 000	354	100	353 ± 6 1
1959 II	8	6 000	354	40	408 ± 14 4
1959 III	7	6 000	354	48	35 ± 8 9
1958 V a	7	7 000	308	20	285 ± 11 8
1958 V b	4	7 000	308	90	307 ± 8 6
1958 V c	3	8 000	267	17	322 ± 9 6

Das Korpergewicht unter Hypoxie zeigt ein Bild wie es in 6 000 m Höhe haufig zu beobachten ist ein initialer Abfall um etwa 15 % der erst nach einer Reihe von Wochen wenn uberhaupt ausgeglichen wird Man hat den Eindruck dass die Tiere ohne Eisenzusatz ein etwas hoheres Gewicht halten die Differenz lasst sich jedoch statistisch nicht sichern

In Tab II sind eine Anzahl weiterer Daten wahrend der verschiedenen Versuchsbedingungen zusammengestellt In keinem Fall finden sich statistisch zu sichernde Differenzen zwischen den beiden Gruppen Das gilt nicht nur fur die Bilanz der Gesamthamoglobinmenge sondern auch fur den Einzelerythrozyten Die Erythrozyten Hb konzentration ausgedruckt durch das Verhaltnis von Hb konzentration/Hamatokrit ändert sich nämlich nicht

Auf Grund dieser Versuche erschien es nicht notwendig den Tieren zusatzlich Eisen zu geben

2 *Absolute und relative Blutwerte unter Hypoxie*

Am eingehensten sind die Veranderungen der Gesamthämoglobinmenge untersucht worden wahrend der ersten Zeit im Sauerstoffmangel in 2—3

geuicht von Ratten vor und in Unterdruck entsprechend 1 000—8 000 m Hohe Die Buchstaben gesetzte Gruppen Der den verschiedenen Höhenstufen entsprechende Barometerdruck entspricht der

Gewicht, g	Tot. Hb g		Tot Hb g/100 g Gew	
In Hypoxie	Vor Hypo	In Hypoxie	Vor Hypoxie	In Hypoxie
380 ± 20 9	2 89 ± 0 122	3 06 ± 0 144	0 76 ± 0 07	0 81 ± 0 073
389 ± 28.2	3 06 ± 0 144	3 11 ± 0 138	0 81 ± 0 073	0.82 ± 0 061
417 ± 7.2	3 08 ± 0 086	3.51 ± 0 105	0 72 ± 0 010	0 84 ± 0 015
403 ± 7 5	3 08 ± 0 086	3.51 ± 0 103	0 72 ± 0 010	0 86 ± 0 073
430 ± 16 9	3 09 ± 0 115	3 64 ± 0 15	0 78 ± 0 029	0.89 ± 0 034
392 ± 31 3	3 05 ± 0 146	4 04 ± 0.2 6	0 76 ± 0 022	1 04 ± 0 039
350 ± 17.2	72 ± 0 104	3 95 ± 0 154	0 73 ± 0 011	1 14 ± 0 035
3 0 ± 19 8	2 35 ± 0 113	3.24 ± 0 187	0 78 ± 0 015	1 01 ± 0 035
300 ± 19 5	3.4 ± 0 187	4.54 ± 0.231	1 01 ± 0 035	1.53 ± 0 097
433 ± 21 5	3 34 ± 0 140	5 15 ± 0.297	0 74 ± 0 014	1 19 ± 0 077
363 ± 15 9	3 64 ± 0 155	6 63 ± 0 473	0 89 ± 0 034	1 84 ± 0 127
267 ± 5 0	1 97 ± 0 074	5.34 ± 0 373	0 67 ± 0 071	2 00 ± 0 135
258 ± 7 6	2 06 ± 0 0 9	4 83 ± 0.237	0 72 ± 0 093	1 87 ± 0 062
84 ± 14.3	2 04 ± 0 090	6.6 ± 0 453	0 67 ± 0 011	2.23 ± 0 165
373 ± 5 8	2 47 ± 0 033	5.37 ± 0.280	0 0 ± 0 014	1 44 ± 0 06
394 ± 11 1	2 67 ± 0 125	5.84 ± 0.264	0 65 ± 0 0 3	1 48 ± 0 048
37 ± 10 0	2.0 ± 0 137	7.37 ± 0 407	0 71 ± 0 077	2.27 ± 0 108
289 ± 15 4	5.4 ± 0 491	5 11 ± 0 447	1 97 ± 0.230	1.8 ± 0.210
304 ± 7 9	4 76 ± 0 750	5.80 ± 0 483	1.56 ± 0.279	1 92 ± 0 199
297 ± 12 9	5 54 ± 0.388	6 88 ± 0 679	1 73 ± 0 170	2 36 ± 0.286

tagigen Abstanden in einer Reihe von Versuchen weiter etwa 2 mal wochentlich Demgegenuber sind die relativen Blutwerte (Hb-konzentration und Hamatokrit) aus denen sich zusammen mit dem Gesamt Hb das Blut Plasma und Erythrozytenvolumen berechnen nicht in allen Hohenstufen gemessen und im allgemeinen auch nicht deren zeitliche Verlaufe verfolgt worden Dadurch sollte eventuellen Blutungen oder Infektionen mit deren moglichen Ruckwirkungen auf das Gesamt Hb vorgebeugt werden

Die Kurvenverlaufe des Gesamt Hb zwischen 2 000 und 5 000 m Hohe weisen im ganzen ein gemeinsames Merkmal auf wie es sich aus Abb 3 deutlich erkennen lasst zunachst steigt das Gesamt Hb je nach dem Grad des Unterdrucks etwas verschieden durchlauft ein Maximum und fällt dann auf ein niedrigeres Niveau ab das etwa dem Wert zwischen 15 und 20 Tag entspricht

Diese Veranderungen hangen nicht damit zusammen dass etwa die Tiere krank geworden sind Eine Erkrankung kann sich zwar auch in einem Abfall oder einer verzogerten Bildung des Gesamt Hb aussern pflegt aber gleichzeitig mit einem Gewichtsverlust verbunden zu sein Ein 24-tagiger Versuch

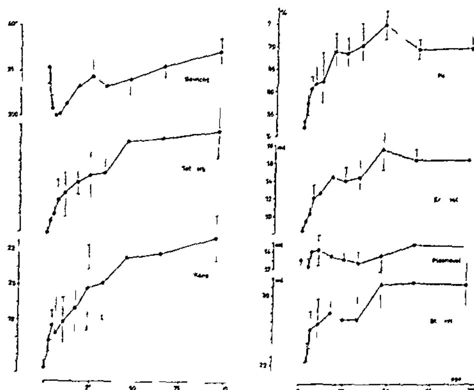


Abb 4 Gewicht und Blutwerte von Ratten in Hypoxie entsprechend 6 000 m H he Mittelwert von 7 Tieren mit Standardabweichung

in 1 000 m zeigte so geringe Veranderungen dass auf seine graphische Darstellung verzichtet wurde

In Tab III sind weiter die Ausgangs- und Endwerte dieser Versuche zusammengestellt Naturlich ist denkbar dass sich die Endwerte bei langerer Versuchsdauer noch etwas verandert hatten sie sind aber besonders wenn das Gesamt Hb auf das Korpergewicht bezogen wird recht instruktiv und lassen die prinzipiellen quantitativen Veranderungen erkennen

Bereits in 1 000 m steigt das Hb signifikant ($p = 0\,01$) auch auf das Korpergewicht bezogen ($p = 0\,05$—$0\,01$) weiter bis zu 3 000 m j doch in relativ bescheidenem Umfang 4 000 m hat bereits eine recht kraftige Wirkung Die Werte lassen sich gut reproduzieren In 5 000 m steigt eine Gruppe stark, eine andere gegenuber 4 000 m kaum Wie spater im einzelnen gezeigt wird hängt diese Differenz mit dem Alter bzw der Grosse der Tiere zusammen

Die relativen Blutwerte zusammen mit dem Blut Plasma und Erythrozytenvolumen von Versuchen in 2 000 4 000 und 5 000 m sind Tab IV zu entnehmen In 2 000 m ist die hohe Hb-konzentration nach 14 tagiger Versuchsdauer bemerkenswert die mit einem hochsignifikanten Abfall ($p <$

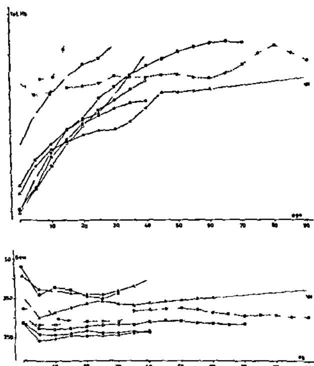


Abb 5 Gew cht und Gesamtl m globin n Ratten n Hypoxi entsp echend 6 000—8 000 m H h Mittelwert in 6 000 m o je 5—8 T er n n 7 000 m von 7—4 T eren in 8 000 m on 3 Tieren Symbol m t V rsuchsbeze chnung ▲—▲ 6 000 m (19 9 I) ●—● 6 000 m (19 8 III) ○—○ 6 000 m (1958 II) — 6 000 m (19 9 II) □—□ 6 000 m (1958 IV) ■—■ 6 000 m (19 7 II b) ○—○ 7 000 (1958 V b) ●—● 8 000 m (1958 V c) D e in Pa enthese ange gebenen Versu hsnummern entsp chen den Bez chnung n in Tab III Folgende Vers hsgruppen befanden h zu o a f iner niedrigeren Unterdruckstuf 19 7 II b 60 T ge n 3 000 m 19 8 V a b 40 T g in 6 000 m 1958 V c 90 T g in 7 000 m

0 001) von Blut und Plasmavolumen verbunden ist Da aber auch gleich zeitig das Gewicht abgefallen ist ($p < 0\,001$) ist die Differenz fur das Blut volumen/100 g Korpergewicht nur schwach signifikant ($p = 0\,05$) Der Versuch wurde nach 4 Wochen mit genau demselben Resultat wiederholt In 5 000 m sind bei dem hohen Gesamt Hb dieser Tiere nicht nur die periphe ren Blutwerte gegenuber 4 000 m kraftig angestiegen sondern auch das Blut volumen ($p < 0\,001$)

Geringerer O_2 Druck als entsprechend 5 000 m Hohe fuhrt durchweg zu einer sehr kraftigen polyzythamischen Reaktion Abb 4 zeigt den Verlauf derartiger Veranderungen in 6 000 m Im wesentlichen haben die Werte nach 40—50 Tagen ein neues Niveau erreicht Das Gesamt Hb und das Erythrozytenvolumen sind etwa verdoppelt bei unverandertem Plasma volumen hat sich das Blutvolumen beinahe um 50 % vergrossert. Der Hamato-

Tab IV Mittelwerte von Hamoglobinkonzentration Hamatokrit Hamoglobinkonzentration × Plasmavolumen von Ratten vor und in Unterdruck entsprechend 2 000—7 000 m Höhe

Fur Körpergewicht und Gesamthämoglobin der Gruppen s. Tab III

Versuch Nr	n	Höhe m	Tage in Hypoxie	Rel Hb g / Vor Hypoxie	Rel Hb g / In Hypoxie	Hct / Vor Hypoxie	Hct / In Hypoxie	Rel Hb g / Hct × 100 Vor Hypox	Rel Hb g / Hct × 100 In Hypoxie
1960 I	9	2 000	14	14 46±0 431	17 60±0 291	47 4±1 73	59 6±1 08	30 5±0 25	29 6±0 07
1960 I	9	2 000	28	—	18 38±0 374	—	60 9±1 23	—	30 2±0 35
1958 Ia	6	4 000	68	—	18 72±0.291	—	61 0±0 63	—	30 7±0 41
1958 Ib	6	5 000	63	18 72±0.291	22 34±0 265	61 0±0 63	70 8±1 20	30 7±0 41	31 6±0 44
1958 II	*6*	*6 000*	*40*	*13 01±0 280*	*24 78±0 709*	*42 0±1 12*	*78 3±1 41*	*31 0±0 16*	*31 6±0 [illegible]*
1958 III	8	6 000	40	12 73±0.228	24 40±0 336	40 6±1 00	74 7±1 54	31 4±0 22	3[illegible] 7±0 44
1958 IV	6	6 000	73	—	24 60±0 572	—	80 3±2 32	—	30 6±0 38
1959 I	7	6 000	100	15 25±0 130	22 69±0 502	52 1±0 57	70 7±1 42	29 3±0.34	31 8±0 28
1959 II	8	6 000	40	14 30±0 233	23 56±0 470	46 2±0 83	76 0±1 15	31 0±0 58	31 0±0 39
1959 III	7	6 000	48	13 35±0 330	26 44±0 422	45 4±1 10	85 3±1 75	29 5±0 7[illegible]	31 0±0.25
1958 Vb	4	7 000	90	21 38±1 630	23 60±1 000	70 0±4 18	75 0±3 37	30 4±0 73	31 6±0 65

krit ist in diesem Versuch etwas rascher als die Hb konzentration gestiegen Das hat zur Folge dass die durchschnittliche Erythrozyten Hb konzentration ausgedruckt durch das Verhältnis von Hb konzentration × 100/Hamatokrit von 29 3 ± 0 34 vor einsetzendem Unterdruck auf 26 9 ± 0 63 nach 17 Tagen Unterdruck hochsignifikant abgefallen ist ($p < 0\ 001$) Im weiteren Verlauf steigt der Quotient aber langsam auf einen Endwert von 31 8 ± 0 82 der sich nicht vom Ausgangswert unterscheidet Dieser Abfall der auf eine verzogerte Hb synthese gegenuber der Zellneubildung hinweisen konnte wurde in keinem anderen Versuch beobachtet

In Abb 5 sind die Veränderungen der Gesamthamoglobinmenge und des Gewichts der Versuche in 6 000—8 000 m dargestellt deren Ausgangs und Endwerte ausserdem Tab III zu entnehmen sind

In 6 000 m kann bis zu einer 3 fachen Gesamtmenge Hb gebildet werden nach 20 Tagen sind etwa 50 % nach 40 Tagen etwa 90 % des Endwertes erreicht Ein Maximum wie in den Versuchen bis zu 5 000 m wird nicht beobachtet Dabei muss dahingestellt bleiben ob ein solches bei längerer Versuchszeit doch noch zu erkennen gewesen wäre Die deutlichen aus Tab III zu erkennenden Differenzen zwischen verschiedenen Tiergruppen werden unten genauer analysiert sie lassen sich mit einer Ausnahme (1959 III) auf Altersfaktoren bzw die Tiergrösse zuruckfuhren

Das Gewicht sinkt in 6 000 m initial um 10—15 % und erreicht nur in einigen Fallen und nach längerer Zeit wieder das Ausgangsniveau Bis auf

100/Hamatokrit Blutvolumen Blutvolumen/100 g Korpergewicht Erythrozytenvolumen und

Blutv l ml		Blutvol ml/100 g Gew		Erythr vol ml		Plasmavol. ml	
V r Hypoxi	In Hypoxie	Vor Hypoxie	In Hypox e	Vo Hypoxie	In Hypo ie	Vor Hypox	In Hypox e
28 6±0 93	26 6±0 99	6 7±0.23	6 4±0 15	10 1±0 33	11 9±0 36	18 4±0 90	14 8±0 61
—	25 4±0 65	—	6.2±0 11	—	11 6±0 3	—	13 8±0.26
—	?3 0±1 17	—	7.2±0.24	—	10 5±0 61	—	1? 5±0.58
23 0±1 17	27 1±1.56	7.2±0.24	9 1±0 52	10 5±0 61	14 4±0 97	12.5±0 58	12 6±0 63
20 2±0 41	26 6±0 73	6.9±0 12	9 7±0 31	6 4±0.27	15 6±0.58	13 8±0.22	11 0±0 43
21 6±0 81	26 6±1 09	7 6±0.32	10 3±0.27	6 6±0 19	15 0±0 7?	15 1±0 68	11 6±0 48
—	33 7±1 79	—	12 0±0 73	—	20 4±1.5?	—	13 3±0 43
21 6±0 25	31 4±1 01	6 1±0 13	8 4±0.20	8 4±0 13	16 7±0 81	13 1±0 18	14 8±0.37
24 9±1 15	33 0±1 37	6 1±0 19	8 4±0 19	8 6±0 45	18 8±0 8?	16 2±0 76	14 2±0 68
25 1±1 53	37 1±1 64	7 1±0 3	11 4±0 47	8 5±0 56	23 8±1 39	16 6±1 0?	13 3±0 48
29.2±2 9	32 5±1 50	9 [illegible]±0 95	10 8±0 68	15 6±2.28	18 3±1 50	13 7±0 70	14 ±0 74

die ersten Tage in Hypoxie waren jedoch Fresslust und Allgemeinbefinden der Tiere nicht beeinträchtigt

Die Tab IV zu entnehmenden peripheren Blutwerte in 6 000 m sind durchweg sehr hoch in einer Versuchsgruppe (1959 III) beträgt der mittlere periphere Hämatokrit 85 % in Einzelfällen sind Werte von über 90 % gefunden worden Im ganzen scheint die durchschnittliche Hb-konzentration der Erythrozyten (Hb konzentration × 100/Hämatokrit) gegenüber den Normalwerten etwas höher zu sein die Differenz lässt sich jedoch statistisch nicht sichern Das Blutvolumen steigt um etwa 20—50 % ($p < 0.001$) wobei das Plasmavolumen im allgemein n um etwa 10—20 / absinkt

Während bis zu 6 000 m Höhe die Tiere direkt dem gewünschten Hypoxiegrad ausgesetzt werden konnten und sich nur in einigen speziellen Untersuchungen auf die später näher eingegangen wird zuvor auf einer geringeren Unterdruckstufe befanden endeten alle Versuche Tiere direkt 7 000 m auszusetzen mit dem Tod dieser Tiere nach einem oder mehreren Tagen Es war also notwendig diese Tiere zunächst an eine niedrigere Höhenstufe zu adaptieren Aber selbst von Ratten die an 6 000 m voll akklimatisiert waren war di Sterblichkeit sehr hoch Das verfügbare Material ist deshalb bescheiden 7 000 m überlebten bis zu 20 Tage 7 Tiere bis zu 30 6 und bis zu 90 Tage 4 Tiere 8 000 m überlebten nur 3 Tiere für 17 Tage

In 7 000 m sinkt das Gesamt Hb abgesehen von einem geringeren initialen Abfall nicht im ganzen findet sich vielmehr eine steigende Tendenz Auch

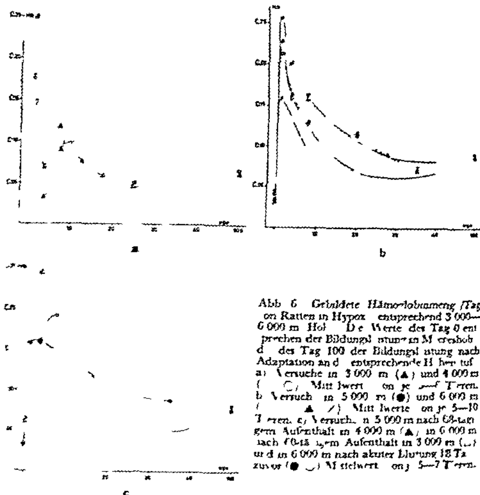


Abb 6 *Gebildete Hämoglobinmeng /Tag* on Ratten in Hypox entsprechend 3 000—6 000 m Hoh De Werte des Tag 0 ent prechen der Bildungsl istung in M eresboh d des Tag 100 der Bildungsl istung nach Adaptation an d entsprechende H hen tuf a) Versuche in 3 000 m (▲) und 4 000 m (◯) Mitt lwert on je —f Tieren. b Versuch in 5 000 m (●) und 6 000 m (▲ /) Mitt lwerte on je 5—10 T eren. c) Versuch n 5 000 m nach 68-tag gem Aufenthalt in 4 000 m (▲) in 6 000 m nach 70-tä igem Aufenthalt in 3 000 m (◡) und in 6 000 m nach akuter Blutung 18 Ta zuvor (● ◡) M ttelwert on j 5—7 Tieren.

das Gewicht verandert ich kaum. Der Tod pflegt plotzlich gelegentlich durch eine akute Blutung oder auch ohne ausserlich sichtbaren Grund einzutreten Die peripheren Blutwerte sind gegenuber 6 000 m praktisch un erandert Der 8 000 m Versuch lasst weitgehendere Schlusse kaum zu Trotz des fallenden Gewichts zeigt das Gesamt Hb kraftig

3 *Die Hämo lobin Bildungsgeschwindigke t unter Hypoxie*

Das im vorangegangenen Kapitel beschriebene Verhalten der Gesamt hamoglobinmenge ermittelt bereits eine gewisse Vorstellung uber die Bildungsleistung der Tiere im Sauerstoffmangel. Einen tieferen Einblick in die Dynamik der Hb-bildung gewinnt man jedoch dann wenn man die taglich gebildete Hb-menge verschiedener Zeitabschnitte v n Hypoxie berechnet D e Ergebnisse derartiger Berechnungen denen die gefundenen Gesamthamoglobinwerte und

eine angenommene Erythrozytenlebenslange von 60 Tagen zugrunde liegen die nach FRYERS und BERLIN (1952) auch unter Hypoxie gilt sind aus Abb 6 ersichtlich Je nach Hypoxiegrad und Ausgangslage der Blutbildung werden dabei drei verschiedene Bildungsverlaufe gefunden

In *massiger Hypoxie* entsprechend 3 000 und 4 000 m Hohe (Abb 6 a) steigt die Hb bildung innerhalb der ersten 48 Stunden auf das etwa 4-fache der Norm/Tag und bildet ein scharfes Maximum fällt zwischen 2 und 5 Tag auf ein Minimum ab um dann zwischen 6 und 20 Tag mit einem ziemlich aus gepragten Maximum um den 10 Tag herum die Masse des Hb zu synthetisie ren Die am 100 Tag eingezeichneten Werte entsprechen der taglichen Bildungsleistung die notwendig ware um die Gesamthamoglobinmenge auf einem 3 000 bzw 4 000 m Hohe entsprechendem Niveau zu halten Die Kurvenverlaufe mussen weiterhin um den 70 Tag herum gedampft die Veranderungen des 10 Tages wiederholen dem Zeitpunkt zu dem die neu gebildeten Zellen ziemlich rasch abgebaut werden

In *kraftiger Hypoxie* entsprechend 5 000 und 6 000 m Hohe (Abb 6 b) ergibt sich ein anderer Bildungstyp Zwischen Tag 0 und Tag 2 findet sich ebenfalls ein scharfes Maximum von etwa gleicher Hohe wie in 3 000 und 4 000 m Hohe Danach fällt die Hb-bildung beinahe kurvenlinear bis zum 40 Tag ab ohne dass ein Minimum um den 4 Tag durchlaufen wird Die starkste Hb-synthese findet hier also bis zum 10 Tag statt die dann wiederum um den 60 Tag erhebliche Ausmasse annehmen muss

Kraftige Hypoxie bei bereits *massig stimulierter Erythropoiese* fuhrt zu einem von diesen beiden beschriebenen Kurventypen deutlich abweichenden Verhalten (Abb 6 c) Allen Versuchen gemeinsam findet sich namlich kein Maximum zwischen Tag 0 und 2 In der Versuchsserie mit dem geringsten Hohenunter schied bei der die Tiere nach etwa 2 monatigen Aufenthalt in 4 000 m auf 5 000 m gebracht wurden ist die durchschnittliche tagliche Hb bildung zu diesem Zeitpunkt nur verdoppelt und erreicht erst nach 2 bis 5 Tagen ein schwaches Maximum Werden Tiere nach etwa 2 Monaten in 3 000 m auf 6 000 m Hohe gesetzt nahert man sich mehr den Verhaltnissen eines unmittel baren 6 000 m Versuchs Der Bildungsverlauf entspricht auch diesem mit der Einschrankung dass das Maximum zwischen Tag 2 und 5 liegt

Ein diesen Versuchen ahnliches Bild erhalt man ebenfalls wenn man die Erythropoiese vor einsetzender Hypoxie auf andere Weise stimuliert In zwei Versuchsreihen wurde den Tieren akut etwa 1/3 der Blutmenge entnommen Unmittelbar nach Abschluss der Regeneration wurden dann die Tiere 6 000 m Hohe ausgesetzt Auch hier fehlt das initiale Maximum und die starkste Hb-synthese erfolgt ebenfalls um etwa den 5 Tag

4 *Der Einfluss von Alter bzw Tiergrosse auf die Blutwerte unter Hypoxie*

Vergleicht man in Tab III das auf das Korpergewicht bezogene Gesamt Hb der verschiedenen Versuchsgruppen in 5 000 und 6 000 m lassen sich

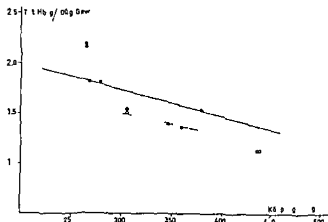


Abb 7 Verhalten der Gesamthämoglobinmenge/100 g Körpergewicht in Abhängigkeit vom Körpergewicht nach 50—60 Tagen Hypoxie entsprechend 5 000 m Höhe und 40 Tagen Hypoxie entsprechend 6 000 m Höhe ○ = 5 000 m Gleichung der Regressionslinie y = — 0 00?13 x + 2 1374 r = — 0 73 p = 0 01—0 001 ● = 6 000 m Gleichung der Regressionslinie y = — 0 00?69 x + ?5498 r = — 57 p < 0 001

deutliche Differenzen feststellen Zunachst wird man die variierende Versuchsdauer dafur verantwortlich machen Ein anderer Faktor mag eine gewisse Inhomogenitat des Tiermaterials darstellen wie sie eine mehrjahrige Versuchsdauer erwarten lasst Schliesslich wird man aber auch an das Alter der Tiere zu denken haben Bereits unter Normalbedingungen finden sich deutliche Unterschiede des Gesamt Hb zwischen jungen und alten Tieren 250 g schwere etwa 50 Tage alte Ratten haben durchschnittlich 0 76 g Hb/100 g Korpergewicht 400 g schwere etwa 200 Tage alte Tiere nur noch etwa 0 71 g Hb/100 g Korpergewicht (TRIBUKAIT 1960 b)

Derartige Differenzen der Hb menge verschieden grosser Tiere lassen sich auch unter Hypoxie nachweisen Das geht aus Abb 7 hervor wo das Gesamt Hb/100 g Korpergewicht auf der Ordinate gegen das Korpergewicht auf der Abszisse von Tieren in 5 000 und 6 000 m aufgetragen worden ist Von den Versuchen in 5 000 m wurden die Werte zwischen 50 und 60 Tag verwendet von den in 6 000 m die Werte des 40 Tages Obwohl der Akklimatisationsprozess in 6 000 m Hohe zu diesem Zeitpunkt noch nicht voll abgeschlossen ist sind die Werte doch untereinander vergleichbar Die Gruppe 1959 III Tab III deren Werte nur einmal am 48 Tag festgestellt worden sind und die vollig aus dem Bild herausfallen wurde ausgeschlossen Das Gesamt Hb/100 g Korpergewicht fallt in beiden Hohenstufen mit steigendem Korpergewicht signifikant ab

Auch die Hb-konzentration sinkt mit steigendem Korpergewicht etwas Die von 29 Tieren nach etwa 40 tagigem Aufenthalt in 6 000 m Höhe berechnete Regressionslinie fur die Hb-konzentration auf der Ordinate gegen das Gewicht auf der Abszisse lautet y = — 0 00971 x + 26 68 Die Hb-kon

zentration von 250 g schweren Tieren betragt danach durchschnittlich rund 24 2 g% die von 400 g schweren Tieren 22 8 g % Die Streuung ist aber gross und der Korrelationskoeffizient hat nur einen Wert von — 0 37 mit einer Signifikanz von p = 0 05

Das aus der Hb konzentration dieser Tiere und dem entsprechenden Gesamt Hb berechnete Blutvolumen fällt ebenfalls mit steigendem Korpergewicht relativ ab das 250 g schwere Tier hat ein Blutvolumen/100 g Körpergewicht von 10 3 ml das 400 g schwere Tier von 8 1 ml Die Gleichung der Regressionslinie lautet y = — 0 0144 × + 13 79 Der Korrelationskoeffizient betragt — 0 72 und ist hochsignifikant (p < 0 001)

Diskussion

Es ist seit langem bekannt dass O Mangel und Blutbildung in einem Zusammenhang stehen Welcher Art dieser Zusammenhang ist ist indessen weitgehend unklar Ein Schritt in der Analyse dieses Problems besteht darin quantitativ den Grad der Erythropoiese in Abhängigkeit vom O_2 Druck zu untersuchen Da relative Blutwerte nur einen beschrankten Einblick in die Erythropoiese geben wurde in den vorliegenden Untersuchungen vorwiegend die Gesamthamoglobinmenge gemessen

Erste Zeichen einer erhohten Erythropoiese lassen sich bereits in Hypoxie entsprechend 1 000 m Unterdruck nachweisen Fur den Menschen gibt VERZÁR (1947) als untere Reaktionsgrenze etwa 2 000 m an

Bis zu 4 000 m Hohe steigt das Gesamt Hb der Ratte in relativ massigem Umfang erreicht dann aber zwischen 4 000 und 6 000 m sprunghaft einen 2—3 fachen Wert Dieser drastische Anstieg spielt sich bei einer arteriellen O Druck Differenz von etwa 10 mm Hg ab darauf wird im Einzelnen im Zusammenhang mit der Analyse der arteriellen Blutgase unter Hypoxie spater eingegangen (TRIBUKAIT 1962 a) 7 000 m und 8 000 m Hohe haben gegen uber 6 000 m eine gewisse weitere Steigerung zur Folge fuhren also nicht zu einem Abfall wie ihn REYNAFARJE (1958) auf Grund der Untersuchungen von TALBOTT (1936) sowie HURTADO MERINO und DELGADO (1945) diskutiert hat

Es ist einigermassen schwierig Daten der Hb bzw Erythrozytenmenge der Literatur mit den eigenen die mit dem Alter der Tiere und von Gruppe zu Gruppe variieren konnen vergleichen zu wollen Von Hohen uber 5 000 m liegen nur wenige Untersuchungen vor REISSMANN (1951) fand beim Hund nach einem Aufenthalt von 12—14 Wochen in 350 mm Hg ein gegenuber der Norm um 70 % erhohtes Erythrozytenvolumen Die eigenen Werte liegen durchschnittlich bei etwa 150 % Aus den von FRYERS (1952) fur Ratten angegebenen allerdings nicht eindeutigen Ziffern lasst sich ein um etwa 140 % hoheres Erythrozytenvolumen als normal errechenen Die Tiere waren 35 Tage lang in 427 mm Hg und 13 Tage in 350 mm Hg

In niedrigerer Höhe entsprechend etwa 430 mm Hg ergaben die umfangreicheren Untersuchungen an Höhenbewohnern der Anden ein gegenüber Meereshöhe um etwa 50 % grösseres Erythrozytenvolumen (HURTADO et al 1945 MERINO 1950 REYNAFARJE LOZANO und VALDIVIESO 1959) Beim Hund in gleicher Höhe scheinen ähnliche Werte vorzuliegen (ROTTA zit von HURTADO et al 1945) Demgegenüber ist das Erythrozytenvolumen von Ratten auch in dieser Höhe offenbar grösser FRYERS (1952) gibt ein etwa 80 % höheres als normal an die eigenen Werte liegen in etwa gleicher Grössenordnung

Aus diesem Vergleich geht hervor dass die Reaktionsweise der Erythropoiese verschiedener Säuger auf O_2 Mangel nicht gleich zu sein scheint Eine Voraussetzung dafür die Ursachen dieser Unterschiede festzustellen ist dass man das Regulationsziel der gesteigerten Erythropoiese unter Hypoxie kennt Wie in einer späteren Arbeit gezeigt wird hängt dieses teilweise mit dem O_2 Gehalt des arteriellen Blutes unter Hypoxie d h den O_2 Transportverhältnissen zusammen (TRIBUKAIT 1962 a)

In 6 000 m Höhe nähert sich die Hb menge offenbar einem oberen Grenzwert Bei der Diskussion der Frage welche Faktoren im Einzelnen die Gesamtmenge Hb eines Organismus begrenzen wird man zunächst zu prüfen haben ob die Bildungsleistung ihren Maximalwert erreicht hat Diese muss über der Abbauquote liegen damit überhaupt eine grössere Menge erreicht werden kann Aus der Berechnung der täglichen Hb synthese ergibt sich dass in 6 000 m zumindest während einer Zeitspanne von 14 Tagen so viel Hb gebildet wird dass diese einer etwa 4 fachen Gesamtmenge Hb genügen würde wenn sie sich auf einer gleichbleibenden Höhe hielte

Nun unterscheiden sich die Verhältnisse in 6 000 m von denen in grösseren Höhen wesentlich In 6 000 m gelingt es nämlich noch die niedrige O_2 Sättigung des Blutes durch eine höhere Hb-konzentration zu kompensieren — offenbar eines der Anpassungsziele unter Hypoxie (TRIBUKAIT 1962 a) Das ist in 7 000 m definitiv nicht mehr möglich Die mit der Zeit abnehmende Bildungsleistung in 6 000 m kann man wie in niedrigeren Höhen durch den mit fortschreitender Hb bildung immer geringer werdenden Bildungsreiz zu erklären versuchen Dass in 7 000 m Höhe trotz eines erniedrigten O_2 Inhalts ebenfalls nur eine gewisse Menge Hb gebildet wird muss also eine andere Ursache haben

Eine direkte schädigende Wirkung zu niedrigen O Drucks auf das Knochenmark ist nach Untersuchungen an Knochenmarkkulturen denkbar (ROTH und RACHMILEWITZ 1948 ASTALDI BERNARDELLI und REBAUDO 1953 BERNARDELLI 1959) Die in vitro untersuchte Protoporphyrin und Hämsynthese sinkt ebenfalls in stärkerem O_2 Mangel ab (FALK et al 1959) Weiter mag der sich verschlechternde Allgemeinzustand auch auf die Erythropoiese negativ einwirken Es sind aber auch aktive gegenregulatorische Prozesse denkbar die dann einsetzen wenn beispielsweise die grosse Blutmenge

oder der steigende Hämatokrit andere Körperfunktionen zum Erliegen zu bringen drohen Endlich ist aber auch vorstellbar dass der O_2 Bedarf in 7 000 m auf andere Weise z B durch eine erhöhte Kapillarisation oder Durchblutung befriedigt wird und damit kein erhöhter Bildungsreiz mehr besteht Der weitere Anstieg in 8 000 m deutet nicht auf ein Versagen der Hb bildung hin und mag diese Vorstellung stützen

Die Frage nach begrenzenden Faktoren bei der O_2 Mangel Polyzythämie interessiert schliesslich auch im Zusammenhang mit der Polycythämia vera bei der 2—3 fache Gesamtmengen Hb nicht selten beobachtet werden (ENGSTEDT FRANZÉN und TRIBUKAIT 1962 a)

Es ist einer der wesentlichen Vorteile der verwendeten CO Methode vom gleichen Tier die Gesamthämoglobinmenge beliebig oft bestimmen und somit deren Bildungsverlauf studieren zu können Bei der Betrachtung der Veränderungen der Hb menge darf man nun nicht übersehen dass diese wenn auch überwiegend so doch nicht vollständig aus zirkulierendem und zu einem kleineren Teil aus nichtzirkulierendem in den Geweben liegendem Hb besteht Wie oben besprochen (s Methodik) kann angenommen werden dass beim normalen Tier etwa 4 % des Gesamt Hb in den Knochenmarkzellen fixiert sind und weitere 2—3 % in dem Retikulozytendepot des Knochenmarks liegen Diese Zellen können in die Zirkulation übertreten gehören also nur fakultativ zum nicht zirkulierenden Hb Das Verhältnis von zirkulierendem zu nichtzirkulierendem Hb lässt sich praktisch dadurch prüfen dass man gleichzeitig das Gesamt Hb mit CO und das zirkulierende Hb mit einer Zellverdünnungsmethode misst In ziemlich guter Übereinstimmung mit dem berechneten Wert wurden für das normale Tier mit einer durchschnittlichen Gesamtmenge Hb von 2 41 g 0 21 g d h etwa 9 % nichtzirkulierendes Hb gefunden (ENGSTEDT PERIC und TRIBUKAIT 1960)

Die hohen Anforderungen an das erythropoietische Gewebe unter Hypoxie lassen bedeutende Verschiebungen des Verhältnisses zwischen zirkulierendem und nichtzirkulierendem Hb erwarten besonders solange sich kein endgültiges Gleichgewicht zwischen Zellneubildung und abbau eingestellt hat Ein Extremfall liegt während der ersten beiden Tage unter Hypoxie vor wo eine kräftige Zellneubildung in Knochenmark und Milz einsetzt (SAATHOFF 1950 a MÖLLER 1960) und gleichzeitig die Retikulozyten des Knochenmarks verdreifacht werden (SAATHOFF 1950 a) ohne dass Retikulozyten im zirkulierenden Blut auftreten Diese Phase der Hypoxie wird noch in einer besonderen Arbeit eingehender besprochen (TRIBUKAIT 1962 b)

Diese Vorgänge durch die das erythropoietisch aktive Gewebe mit Hb gewissermassen aufgeladen wird finden ihren deutlichen Niederschlag in einem initialen Hb maximum wenn man die täglich gebildete Hb menge unter Hypoxie zur Darstellung bringt (s Abb 6) Die Höhe dieses Maximums d h also die Quantität Hb die in den blutbildenden Geweben zu liegen kommt zeigt nur eine schwach ausgeprägte Abhängigkeit vom Grad des

Unterdrucks Ein solches Maximum ist nicht oder nur in geringerem Umfang bei bereits stimulierter Erythropoiese zu erwarten Dabei sind Hypoxie und Anämie als Stimulus wie sich zeigt etwa gleich wirksam Der geringste Effekt liegt dann vor wenn die *Differenz zwischen der Ausgangslage der Erythropoiese* und dem Zusatzreiz gering ist (z B zwischen 4 000 und 5 000 m) umgekehrt ist die Differenz z B zwischen 3 000 und 6 000 m gross und entsprechend auch der initiale Hb-anstieg Es sei hier betont dass die täglich gebildete Hb-Menge die sich aus dem gefundenen Gesamt Hb und einer angenommenen Erythrozytenlebenslänge von 60 Tagen errechnet als Mindestwert anzusehen ist FRYERS und BERLIN (1952) haben zwar unter Hypoxie eine im wesentlichen unveränderte Erythrozytenlebenszeit gefunden Ob das aber auch in 6 000 m Höhe bei extrem hohen Hämatokritwerten der Fall ist muss offen bleiben

In diesem Zusammenhang taucht nun nicht nur die Frage auf welche Prozesse die Zellbildung sondern auch den Abstrom der gebildeten Zellen in die Zirkulation steuern und wie diese beiden Prozesse miteinander verknüpft sind Es erscheint gesichert dass der Zellausstoss aus den erythropoietischen Geweben unter Hypoxie zunächst nicht kontinuierlich vor sich geht und nicht zur Zellbildung in einer einfachen Proportion steht Zwischen dem hypoxischen Reiz und der darauffolgenden Retikulozytensteigerung im Blut liegt eine Latenz die auch mit der Dauer und dem Grad der Hypoxie variiert (SUNDSTROEM und MICHAELS 1942 SAATHOFF 1950 b MERTENS 1957 MOLLER 1960) Es ist vorstellbar dass die Durchblutung des erythropoietischen Gewebes in diese Beziehungen mit wesentlich eingreift Weitere denkbare Faktoren werden in einer anderen Arbeit diskutiert (TRIBUKAIT 1962 b) Die Zellneubildung bis zu 4 000 m Höhe geht soweit sich diese aus dem Verlauf der täglich gebildeten Hb menge beurteilen lässt ebenfalls nicht kontinuierlich vor sich

Einen relativen Abfall der nichtzirkulierenden Hb menge wird man andererseits dann erwarten dürfen wenn mit grosser werdender Gesamtmenge Hb die Anforderungen an die Leistung des blutbildenden Gewebes geringer werden Die mehr oder minder ausgeprägten Maxima der Kurvenverläufe der Gesamthämoglobinmenge bis zu 5 000 m Höhe die sich dann je nach dem O_2 Druck auf ein gewisses Niveau einstellen können in dieser Richtung gedeutet werden und nicht als eine überschiessende Bildung von zirkulierendem Hb Dass in 6 000 m keine derartigen Maxima mehr gefunden werden mag sich daraus erklären dass die weiterbestehenden Forderungen an das *blutbildende Gewebe dessen Einschränkung nicht zulassen* Die nichtzirkulierende Hb-menge von Ratten nach mehrwöchentlichem Aufenthalt in 6 000 m berechnet aus dem mit CO und Cr^{51} gezeichneten Erythrozyten bestimmten Hb betrug bei einer durchschnittlichen Gesamtmenge von 5 65 g 0 7 g d h also etwa 3 mal soviel wie normal (ENGSTEDT *et al* 1960)

Aus dem Angeführten folgt dass die aus dem Gesamt Hb und den relativen Blutwerten berechneten Blut bzw Plasmavolumina mit einem Unsicher

heitsmoment behaftet sind Es kann aber kein Zweifel daruber bestehen und alle diesbezuglichen Untersuchungen stimmen darin uberein dass das Blut volumen bei langanhaltendem Hohenaufenthalt mehr oder minder kraftig ansteigt (HURTADO *et al* 1945 MERINO 1950 REISSMANN 1951 FRYERS 1952) Bei den vorliegenden Untersuchungen bildet nur der 2 000 m Versuch eine Ausnahme davon hier findet sich namlich ein geringeres Plasmavolumen ohne ein entsprechend grosseres Erythrozytenvolumen Daraus resultiert eine relativ hohe Hb konzentration des Blutes die im Sinne einer Hamokon zentration zu deuten ist

Betrachtet man das Blutvolumen als Mass der aktuellen Grosse des Gefäss systems bedeutet ein 30—50 % grosseres Blutvolumen wie es hier gefunden wird eine wesentliche Dimensionsanderung des Gefassystems Die von MERCKER und SCHNEIDER (1949) MERCKER und OPITZ (1949) OPITZ (1952) sowie VALDIVIA (1956) beschriebene erhohte Kapillarisation unter Hypoxie stellt die funktionell wichtigste Seite dieser Dimensionsanderung dar MERCKER und OPITZ (1949) messen dieser Zunahme der Kapillarisierung eine weitaus grossere Bedeutung fur die O Versorgung der Zellen als der hoheren Hb-konzentration oder einer Durchblutungssteigerung zu Aber auch andere Abschnitte des Gefassystems nehmen an dieser Dimensionsanderung teil das von der Ratte rontgenologisch in vivo bestimmte Herzvolumen steigt mit dem Blutvolumen Die gute und den normalen Verhaltnissen entsprechende Korre lation zwischen Blutvolumen und Herzvolumen spricht weiterhin dafur dass die Dimensionsanderung der verschiedenen Gefässabschnitte in gesetzmassiger Weise erfolgt (TRIBUKAIT 1962 c) Das ist wie das unveranderte Herzvolumen bei der Polycythamia vera zeigt durchaus keine Notwendigkeit (ENGSTEDT FRANZEN und TRIBUKAIT 1962 b)

Daruber wie Altersfaktoren bzw die Tiergrosse zu einer verschiedenen hypoxischen Reaktion fuhren die das Gesamt Hb ebenso wie die Hb-kon zentration und das Blutvolumen betrifft lassen sich nur Vermutungen aus sern Das gefundene Bild ist jedoch dem Verhalten von thyreoidektomierten und normalen Tieren in Hypoxie (VAN DYKE *et al* 1954) so ahnlich dass d e Vermutung nahe liegt dass auch hier Anderungen im Stoffwechsel von Bedeutung sind VAN DYKE *et al* fanden im Normaldruck keine Unterschiede der peripheren Blutwerte und geringere Differenzen der Gesamthamoglo binmenge zwischen normalen und thyreoidektomierten Tieren unter Hypoxie geringere Unterschiede der Hb-konzentration und kraftigere des Gesamt Hb

Die vorliegenden Untersuchungen kommen zwar dem Kernpunkt der Frage in welchen Zusammenhang O Druck und Hamoglobinbildung stehen und welchem Regulationsziel die Erythropoiese unter variierendem O Druck folgt naher Einige zur weiteren Analyse dieses Problems wesentliche Grossen der arterielle O Druck und die arterielle O Hb-Sattigung fehlen hier jedoch Darauf soll in einer spateren Arbeit eingegangen werden (TRIBUKAIT 1962 a)

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Circulatory Studies in Well Trained Athletes at Rest and During Heavy Exercise, with Special Reference to Stroke Volume and the Influence of Body Position

By

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R ceived 2 J ne 19??

Abstract

Bevegård S A. Holmgren and B Jonsson *Circulatory stud es in well trained athletes at rest and during heavy exercise with special refer nce to stroke volume and the influence of body position* Acta physiol scand 1963 57 26—50 — Eight well trained athletes (cyclists) with large dimen s ons of the circulatory system were tudied with heart catheteriza tion at rest and during exercise at 800 and 1 600 kpm/min both wh l sitting and supine The results are compared with data from non athletes The card ac output at rest and during work showed the same relation to the oxygen uptake as in nonathletes The cardiac output was less in the sitting than in the supine pos tion by 2 6 l/min at rest and 1 8 l/min during heavy work, du to a smaller stroke volume in the sitting position in both groups On transit on from rest to exerc se the stroke olume increased 9 in the supine and 48 in the s tting position After these initial changes the stroke volume remained con stant during continued exercise with the heavier load in both body positions The difference in stroke volume between supine and sitting position averaged 43 ml at rest and 9 ml during heavy exercise The stroke volume during work in the supine position showed the same relation to the blood volume as in the nonathletes The stroke volume was larger than expected from the size of the heart when compared with the previously demonstrated relationsl ip in nonathletes The rate of work, performed at pulse rat 1/0 bore approximately the same relationsh p to the stroke olume during exercise in both groups The larger oxygen transport capac ty of these athletes as compared to nonathletes is explained by a larger troke volume During exercise the ventricular f lling pressures were higher than in nonathl tes

According to traditional views the cardiac output during exercise in normal subjects is augmented by increases in both stroke volume and heart rate An increase in cardiac output was assumed to depend on displacement of blood to the heart with subsequent increase of diastolic filling and systolic output according to Starling s law of the heart This concept is supported by some observations with indirect estimations of cardiac output by gasometric techniques (KROGH and LINDHARD 1912 LINDHARD 1915 CHRISTENSEN 1931 JØRGENSEN 1934 BRANDI and BRAMBILLA 1961) Although the stroke volume was usually found to be larger during exercise than at rest a continuous increase in stroke volume during exercise was no constant finding (BOOTHBY 1915 DOUGLAS and HALDANE 1922 CHRISTENSEN 1931 NIELSEN 1937) ASMUSSEN and NIELSEN (1952) found no significant difference between values obtained with the Grollman and with the dye dilution technique and their data reveal a marked constancy of the stroke volume during work up to fairly high intensities During recent years a number of investigations with the direct Fick method (RILEY *et al* 1948 DEXTER *et al* 1951 SLONIM *et al* 1954 DONALD *et al* 1955 BARRATT BOYES and WOOD 1957 HOLMGREN JONSSON and SJOSTRAND 1960 BEVEGÅRD HOLMGREN and JONSSON 1960 REEVES *et al* 1961 a b) or the indicator dilution technique (WANG MARSHALL and SHEPHERD 1960 a BRAUNWALD and KELLY 1960) have failed to support the view of an increasing stroke volume during work. This has also been noted in reviews by SJOSTRAND (1956) and by RUSHMER and SMITH (1959) In some of the above mentioned reports and in some other studies where determinations were made only during light work a slight to moderate increase in stroke volume was found in some individuals especially on transition from rest to exercise (WARNER *et al* 1953 VARNAUSKAS 1955 MULLER 1959 MCGREGOR ADAM and SEKELJ 1961) A marked constancy of the stroke volume during exercise has also been demonstrated in dogs (RUSHMER 1959 WANG MARSHALL and SHEPHERD 1960 b)

The observations referred to appeared inconsistent with the results reported by MITCHELL SPROULE and CHAPMAN (1958) and MUSSHOFF *et al* (1959) who found a significant increase of the stroke volume during work Some of the discrepanices in both older and recent literature can be explained by the fact that some studies were made in the supine and some in the sitting position Thus MITCHELL *et al* (1958) examined the subjects in the erect position and then found a two-fold increase of the stroke volume from rest to exercise In all the other studies referred to either the whole procedure was performed in the supine position or if exercise was performed in the sitting position these values were compared with ontrol values at rest in the supine position

It is well documented that at rest transition from supine to erect position causes a decrease of the stroke volume (NYLIN 1934 ASMUSSEN CHRISTENSEN and NIELSEN 1939 MCMICHAEL and SHARPEY SCHAFER 1944 STEAD *et al* 1945 LAGERLOF *et al* 1951 DONALD *et al* 1953) In a recent study of 10 normal subjects the stroke volume was 40 % smaller in the sitting than in the supine

position (BEVEGÅRD *et al* 1960) On transition from rest to exercise it increased to values slightly below those obtained during exercise in the supine position and was then constant as in the supine position during continued exercise with a heavier load This effect of body position during exercise has also been demonstrated by others (CHAPMAN FISHER and SPROULE 1960 WANG *et al* 1960 a REEVES *et al* 1961 a MCGREGOR *et al* 1961)

However some discrepancies in the literature could be due to differences in technique and material With the more accurate techniques of indicator dilution or direct Fick only MUSSHOFF *et al* (1959) have found a significant increase of the stroke volume during work in the supine position Most reports comprise only a few subjects and the selection of the subjects may be different The importance of this factor is indicated by the result obtained by MUSSHOFF *et al* (1959) They found the increase of the stroke volume during work to be 60 % in athletes and 39 % in ordinary males Thus the controversial result in their study of ordinary males as related to other studies could be due to a difference in selection of subjects their normal cases might be more athletic than in the other studies However judging from the available data (heart volume and pulse reaction during exercise) their normal subjects were not more athletic than the ones studied by HOLMGREN *et al* (1960 a) and BEVEGÅRD *et al* (1960) Furthermore other studies of athletes do not show this marked increase In 3 athletes studied with the direct Fick method in the supine position FREEDMAN *et al* (1955) found that the stroke volume increased to 25 % above the resting value with slight work loads but at a higher work load with a pulse rate of 141 beats/min it was only 13 % above the resting value WANG *et al* (1960 c) studied 3 athletes with the dye dilution technique and found that the stroke index was relatively constant during work and only 8—10 % higher at a pulse rate of 180—195 beats/min than at rest in the supine position With the Grollman technique however the results have varied (CHRISTENSEN 1931 JORGENSEN 1954)

This investigation was carried out to get additional data concerning the behavior of the stroke volume during heavy exercise and more information about the mechanism of the high oxygen transport capacity in athletes both in the supine and sitting positions In earlier reports from our hospital the central circulation at rest and during exercise in ordinary subjects has been described The present paper is a report of eight athletes studied with identical methods and procedures

Material

The material consists of 8 very well trained athletes all belonging to the elite of Swedish cyclists They were examined during a period from February to March when they had started to train again for the coming season but according to their own opinion they were in a stage of medium degree of fitness Some anthropometric data of the ma

Table I Some anthropometric data of 8 well trained athletes

Case no	Age years	Height cm	Weight kg	BSA, m²	Heart vol m ml	Total haemoglobin g	Total h moglobin g/kg	Bl volume l	Work intensity kpm/min at pulse rate 170 beats/min				H rt rate (standing) beats/min
									Sitting		Supine		
									Before cath.	During cath.	Before cath.	During cath.	
1	20	177	66	1 84	1 090	8 7	1 .5	6 5	1 600	1,500	1 510	1 570	66
2	19	180	71	1 9?	1 120	1 003	14 1	8 0	1 800	1 810	1 8_0	1 890	0
3	8	175	71	1 87	1 190	1 117	1 7	8.1	1 800	1 940	1 710	1 810	8
4	19	188	75	2 04	1 070	1 115	14 9	8.0	1 790	1 890	1 840	1 750	8
5	17	18?	64	1 8?	9 0	898	14 0	6 5	1 590	1,530	1 640	1 640	87
6	19	174	7?	1 86	1 09	891	12 4	7 3	1 8 0	1 9 0	1 780	1 900	87
7	28	181	75	1 96	1 060	1 015	13 5	7 7	1 5?0	1 60	1 610	1 770	94
8	2?	177	7?	1.90	1 1 0	1 077	15 0	8 0	1 610	1 690	1 770	1 660	110

terial are presented in Table I The subjects were thoroughly examined before the investigation including history examination of the heart and lungs X ray of the chest ECG at rest and during exercise and routine blood and urine analysis All results were normal and all subjects were regarded as being healthy

The results have been compared with earlier published data from two series of normal nonathletic subjects One series (HOLMGREN *et al* 1960 a) comprises 14 male and 4 female subjects examined at rest and during exercise in the supine position The other series (BEVEGÅRD *et al* 1960) of 10 male subjects was examined at rest and during exercise both in the supine and sitting positions In discussing the effect of body position the athletes are compared with the latter subjects but otherwise with both groups The reference group which has been used is indicated in each figure The athletes and the males in the two reference groups are comparable with regard to age and body size

Method and procedure for examination

The methods employed methodological errors and procedure for examinations have been the same as earlier reported by BEVEGÅRD *et al* (1960) Lactate concentration of the arterial blood was determined according to the method of BARKER and SUMMERSON (1941) as slightly modified by STROM (1949 The breathing valve used in this investigation was a Hans Rudolph high velocity valve With this valve a flow rate of 200 l/min can be maintained with a pressure difference of 4 cm H O

Right heart catheterization was performed in the usual manner Cardiac output was determined according to the direct Fick method with analysis of oxygen saturation and hemoglobin concentration of the blood spectrophotometrically (HOLMGREN and PERNOW 1959) The expired air collected in Douglas bags was analysed according to HALDANE and PRIESTLEY (1935 In the supine position the reference level for zero pressure was taken at 5 cm below the insertion of the fourth rib at the sternum. In the sitting position zero pressure was measured at the insertion of the fourth rib at the sternum.

The volunteers were thoroughly informed about the procedures and were familiar with the breathing valve. They had a light meal in the morning but received no premedication before the heart catheterization The subjects were studied both at rest in the supine position and then after approximately 6 min rest while sitting on the bicycle. This was immediately followed by determinations during exercise in the sitting position at two progressive loads 800 and 1 600 kpm/min The subjects then rested in the supine position for 30—45 min After this time interval the procedure was repeated in the supine position at rest and during work at the same loads as in the sitting position. In cases no 2 3 and 5 no determinations were obtained at rest in the sitting position because of imminent syncope Their symptoms rapidly disappeared when exercise started Case no 5 exercised in the supine position before exercising in the sitting position.

Results

The *ECG* at rest recumbent in the standing position and during and after exercise was normal in all cases During heart catheterization a few ventricular premature beats sometimes appeared at the highest work loads both in the sitting and supine positions Only case no 8 showed a marked pulse increase on standing The average pulse rate in the standing position was 85 beats/min (range 66—110)

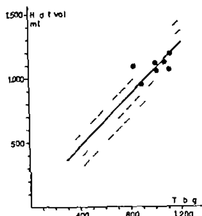
The total amount of hemoglobin (THb) averaged 993 g (range 827—1 117) corresponding to 14 0 g/kg body weight (range 12 4—15 7) which is significantly higher ($p < 0\ 001$) than found in non athletic healthy young men (HOLMGREN *et al* 1957)

The hemoglobin concentration taken as the mean of the samples from the brachial and pulmonary arteries averaged 13 85 g per 100 ml blood (range 12 2—15 6)

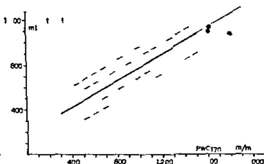
The total blood volume averaged 7 51 l (range 6 5—8 1) corresponding to 105 ml/kg body weight which is significantly higher ($p < 0\ 001$) than found in non athletes (HOLMGREN *et al* 1958)

The heart volume in the prone position averaged 1 087 ml (range 950—1 190) which is larger than in ordinary subjects of about the same body size (BEVEGÅRD *et al* 1960 HOLMGREN *et al* 1960 a) but of the same order as earlier found in athletes (HOLMGREN 1956) When correlated with the total amount of hemoglobin it was in all subjects within the range of two standard errors of estimate from the normal regression line (Fig 1)

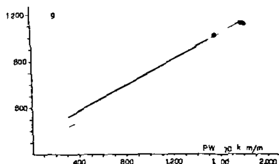
The rate of work performed on a bicycle ergometer at a pulse rate of 170 beats/min (PWC_{170}) was determined both in the sitting and supine positions It averaged 1 700 kpm/min (range 1 560—1 850) in the sitting and 1 701 kpm/min (range 1 510—1 [illegible]) in the supine position Thus there was on the average no difference in PWC_{170} between the two body positions The PWC_{170} was also calculated from the heart rates during exercise at cardiac catheterization and was then 1 761 kpm/min (range 1 500—1 970) in the sitting and 1 756 kpm/min (range 1 520—1 800) in the supine position Thus the capacity to perform muscular work at a given pulse rate was not significantly altered by the procedure



Fg 1



Fg 2



Fg 3

Fg 1 Heart volume n lati n to total amount of hem gl bin (THb) Full lin ind tes th normal re gress n (HOLGREN *et al* 19 7) b oken lines rep esent ± n and two tandard errors f estimat

Fg 2 Heart lum in relati n to the rat of w k t a heart rat of 170 b ts/min (PCW$_{170}$) Regress n l n as in Fg 1

Fg 3 T tal amount of h mogl bin (THb) in r l ti n to r t f wo k at a heart rate f 170 be ts/m n (PCW) Regress on lin as in Fg 1

The PWC$_{170}$ in the sitting position before catheterization was on an average somewhat higher than expected from the normal relationship to heart volume (Fig 2) It was on an average also somewhat higher than predicted from the THb (Fig 3) No subject had a PWC$_{170}$ significantly lower than predicted from the heart volume or THb

Data obtained in connection with heart catheterization

The heart rate at rest in the supine position during the first determination of cardiac output was 63 beats/min (range 55—84) During the second determination in the supine position it was 66 beats/min (range 56—85) In the sitting position after 6—7 min resting on the bicycle it was 64 beats/min (range 58—68) *i e* approximately the same as at rest in the supine position During determination of the basal metabolic rate prior to heart catheterization the heart rate averaged 54 beats/min (range 46—60) The deviations from

Table II. The effect of body position and exercise on some circulatory functions. Mean values of

	PWC_{170} kpm/min		Oxygen uptake ml/min (STPD)			O_2 sat. in mixed ven. blood, per cent		
	Before cath.	During cath.	Rest	Work I	Work II	Rest	Work I	Work II
Recumbent	1 704	1 736	344.6	1 768.9	3 363.9	77.2	47.1	31.4
Sitting	1 700	1 761	384.4	1 864.3	3 386.8	66.8	40.8	27.0
Difference	4	−25	−39.8	−95.4	−22.9	10.4	6.3	4.4
No. of cases	8	8	5	7	8	6	8	8
Standard deviation	77	91	34.1	73.0	103.3	3.5	2.9	1.7
Probability	>0.8	>0.4	>0.05	<0.02	>0.5	<0.001	<0.001	<0.001

PWC_{170} = rate of work at a heart rate of 170 beats/min.

basal conditions are of the same order as earlier found in ordinary subjects (BEVEGÅRD *et al.* 1960).

The heart rate during exercise increased in the supine position to 115 beats/min (range 105—124) on the first load and to 160 beats/min (range 149—175) on the second load. The corresponding figures in the sitting position were 112 beats/min (range 102—132) and 159 beats/min (range 149—175). There was no significant difference in pulse rate at the same load between the two body positions.

The oxygen uptake at rest in the supine position during the first determination was on the average 31.7 % (range 18—47) higher than the predicted basal value (HARRIS and BENEDICT 1919). During the second determination at rest in the supine position it was 40.0 % (range 26—67) and at rest sitting on the bicycle 49.9 % (range 37—66) higher than the predicted basal value. The deviations from the predicted basal values are more marked than in a similar study of ordinary individuals (BEVEGÅRD *et al.* 1960). The oxygen uptake during basal conditions prior to catheterization was on the average 9.6 % higher than the predicted normal value.

The oxygen uptake during exercise increased at 800 kpm/min to an average of 1 864 ml (range 1 758—2 001) in the sitting and 1 769 ml (range 1 650—1,938) in the supine position. At 1 600 kpm/min the values were 3 387 ml (range 3,150—3 666) and 3 364 ml (range 3 088—3 601) in the sitting and supine positions respectively. The difference in oxygen uptake between work in sitting and supine position was probably significant ($p < 0.02$) at 800 kpm/min but insignificant at the heavier load. The mechanical efficiency at the first work load averaged 23.8 % in the sitting and 25.3 % in the supine position. At the second work load it was 24.4 % and 24.6 % respectively. The difference between sitting and supine position was probably significant at the first but

the material and statistical significance of differences

AV-O₂-difference ml/l			Cardiac output l/min			Stroke volume ml		
Rest	Work I	Work II	Rest	Work I	Work II	Rest	Work I	Work II
38 5	94 3	128	9 18	18 98	26 ?6	140 8	163 0	164 1
60 7	110 3	138 4	6 61	16 89	?4 50	10? 6	149 0	154 8
–?2	–16 0	–10 2	2 57	2.09	1 ?6	38	14 0	9 3
6	8	8	5	7	8		7	8
7 8	7 7	5.5	0 89	1 88	1 48	12 3	16 4	9 0
<0 001	<0 001	<0 01	<0 01	<0 05	<0 0?	<0 01	>0 0	<0 0

insignificant at the second work load The mechanical efficiencies were almost the same as those found by MUSSHOFF *et al* (1959) in athletes during similar conditions but somewhat higher than those observed by ÅSTRAND (1952) and HOLMGREN (1956)

The lactate concentration in arterial blood increased during exercise at the highest load to an average of 4 96 meq/l (range 3 8—6 5) in the supine and to an average of 3 36 meq/l (range 1 8—4 6) in the sitting position The difference in lactate concentrations during work between the two body positions is significant ($p < 0\ 001$) The values obtained in the sitting position are of the same order as earlier reported in athletes at corresponding heart rates (HOLMGREN 1956)

The arterial oxygen saturation was on the average 97 8 % (range 95—100) at rest It decreased during work at the first load to 96 1 % (range 94—99) both in the sitting and supine positions and at the heavier load to 95 1 % (range 91—98) in the sitting and to 94 5 % (range 92—97) in the supine position The difference in saturation between the two body positions was not significant The decrease at the highest work load was probably significant ($p < 0\ 05$) and slightly larger than observed in similar subjects at corresponding work loads (HOLMGREN 1956) but of the same order as expected from determinations of arterial oxygen tension during exhaustive work (HOLMGREN and LINDERHOLM 1958) In these earlier investigations the measurements were performed without simultaneous collection of expired air and consequently with out respiratory valves

The oxygen saturation of mixed venous blood at rest as well as during work was lower in all cases in the sitting than in the supine position At rest it averaged 77 2 % (range 74—80) in the supine and 66 8 % (range 6?—73) in the sitting position These values are almost identical with those earlier observed in a

Table II The effect of body position and exercise on some circulatory functions Mean values of

	PWC_{170} kpm/min		Oxygen uptake ml/min (STPD)			O_2-sat in mixed ven. blood per cent		
	Before cath.	During cath.	Rest	Work I	Work II	Rest	Work I	Work II
Recumbent	1 704	1 736	344 6	1 768 9	3 363 9	77 2	47 1	31 4
Sitting	1 700	1 761	384 4	1 864 3	3 386 8	66 8	40 8	27 0
Difference	4	−25	−39 8	−95 4	−22 9	10 4	6 3	4 4
No. of cases	8	8	5	7	8	6	8	8
Standard deviation	77	91	34 1	73 0	103 3	3 5	2 9	1 7
Probability	>0 8	>0 4	>0 05	<0 02	>0 5	<0 001	<0 001	<0 001

PCV_{170} = rate of work at a heart rate of 170 beats/min

basal conditions are of the same order as earlier found in ordinary subjects (BEVEGÅRD *et al* 1960)

The heart rate during exercise increased in the supine position to 115 beats/min (range 105—124) on the first load and to 160 beats/min (range 149—175) on the second load The corresponding figures in the sitting position were 112 beats/min (range 102—132) and 159 beats min (range 149—175) There was no significant difference in pulse rate at the same load between the two body positions

The oxygen uptake at rest in the supine position during the first determination was on the average 31 7 % (range 18—47) higher than the predicted basal value (HARRIS and BENEDICT 1919) During the second determination at rest in the supine position it was 40 0 % (range 26—67) and at rest sitting on the bicycle 49 9 % (range 37—66) higher than the predicted basal value The deviations from the predicted basal values are more marked than in a similar study of ordinary individuals (BEVEGÅRD *et al* 1960) The oxygen uptake during basal conditions prior to catheterization was on the average 9 6 % higher than the predicted normal value

The oxygen uptake during exercise increased at 800 kpm/min to an average of 1 864 ml (range 1 758—2 001) in the sitting and 1 769 ml (range 1 650—1 938) in the supine position At 1 600 kpm min the values were 3 387 ml (range 3 150—3 666) and 3 364 ml (range 3 0?3—3 601) in the sitting and supine positions respectively The difference in oxygen uptake between work in sitting and supine position was probably significant ($p < 0.02$) at 800 kpm/min but insignificant at the heavier load The mechanical efficiency at the first work load averaged 23 8 % in the sitting and 25 3 in the supine position At the second work load it was 24 4 % and 24 6 respectively The difference between sitting and supine position was probably significant at the first but

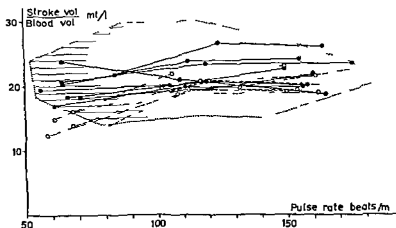


Fig. 6. Stroke volume divided by blood volume in relation to pulse rate at rest and during exercise in supine (filled circles connected with full lines) and sitting position (open circles connected with broken lines). The area within the dotted lines indicates the normal variation obtained from determinations on 27 healthy subjects in the supine position. The shaded area represents the va t o   t   t (HOLMGREN *et al.* 1960 a, BEVEGÅRD *et al.* 1960).

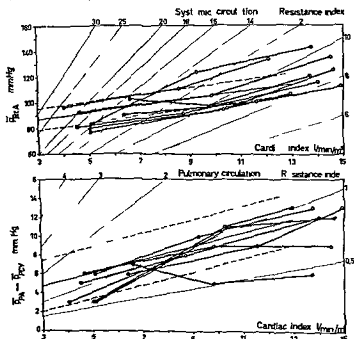


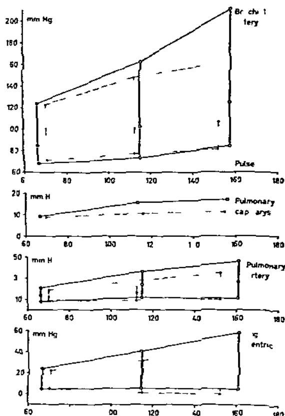
Fig. 7. Mean pressure in brachial artery ($\bar{P}_{BA}$) in relation to cardiac index (C.I.). Pressure gradient over pulmonary vascular bed expressed as the difference between pulmonary arterial mean pressure ($\bar{P}_{PA}$) and mean pulmonary capillary venous pressure ($\bar{P}_{PCV}$) in relation to cardiac index (below). The oblique lines are iso-resistance index lines. The normal regression lines $\pm$ one standard error of estimate are also denoted. Regression equations: $\bar{P}_{BA} = 78.8 + 2.77$ C.I., $r = 0.65$, S.D. $= \pm 8.4$, $n = 86$ and $(\bar{P}_{PA} - \bar{P}_{PCV}) = 53 + 0.72$ C.I., $r = 0.57$, S.D. $= \pm 2.72$, $n = 51$ (BEVEGÅRD *et al.* 1960, HOLMGREN *et al.* 1960 a).

Table III Pressures mm Hg at rest and during exercise

	Rest			Work load I			Work load II		
	$\overline{X}$	SD	n	$\overline{X}$	SD	n	$\overline{X}$	SD	n
Right ventricle									
Systolic									
Recumbent	24.4	2.5	7	40.9	3.8	8	58.5	4.7	8
Sitting	27.1	2.7	7	41.4	4.6	8	60.6	9.0	8
End diastolic									
Recumbent	5.1	2.7	7	5.9	3.7	8	4.6	4.9	8
Sitting	4.0	1.8	7	6.8	3.7	8	6.0	3.3	8
Initial-diastolic									
Recumbent	—0.4	2.4	7	—1.4	3.1	8	—4.1	3.5	8
Sitting	1.0	1.6	7	0.8	3.2	8	—1.1	2.4	8
Pulmonary artery									
Systolic									
Recumbent	20.6	1.6	7	36.0	4.7	8	47.0	5.3	8
Sitting	19.1	3.5	7	33.1	6.4	8	46.3	7.5	8
Diastolic									
Recumbent	7.6	2.7	7	12.4	4.4	8	12.4	6.1	8
Sitting	9.1	1.1	7	14.0	4.0	8	13.1	5.1	8
Mean									
Recumbent	13.6	2.0	7	24.8	1	8	28.6	5.5	8
Sitting	13.0	1.7	7	22.6	4.4	8	27.6	4.8	8
Brachial artery									
Systolic									
Recumbent	123.4	12.1	7	162.4	15.1	8	211.1	27.1	8
Sitting	131.9	24.9	7	174.5	25.8	8	201.8	28.1	8
Diastolic									
Recumbent	68.3	7.1	7	73.5	5.6	8	81.6	7.3	8
Sitting	81.3	11.7	7	83.0	8.0	8	87.0	11.5	8
Mean									
Recumbent	85.2	8.0	6	10[illegible].9	10.5	8	121.5	12.5	8
Sitting	99.7	16.8	6	113.0	11.2	8	119.5	15.7	8

$\overline{X}$ = average, SD = standard deviation n = number of cases

series of ordinary subjects (BEVEGÅRD *et al* 1960) During exercise at 800 kpm/min it decreased to 47.1 % (range 43—54) in the supine and to 40.8 % (range 38—45) in the sitting position At a load of 1 600 kpm/min the values were 31.4 % (range 28—38) and 27.0 % (range 25—31) respectively The differences between supine and sitting posture are significant (Table II) The oxygen saturation of mixed venous blood varied in relation to the pulse rate approximately within the range found for ordinary subjects but was on the



F 8 Mean values in upine position of pressures fr m brachial artery pulmonary arterial wedge position pulmonary artery and right ventricle at rest (filled circles) and during exercise (open circles) Broken lines connect values for normal subjects (BEVEGÅRD *et al* 1960 HOLMGREN *et al* 1960)

average somewhat lower during exercise (BEVEGÅRD *et al* 1960 HOLMGREN *et al* 1960 a)

The arterio venous oxygen difference at rest was on the average 38 5 ml/l (range 34—43) in the supine and 60 7 ml/l (range 51—68) in the sitting position At 1 600 kpm/min the values were 128 2 ml/l (range 123—134) and 138 4 ml/l (range 132—146) respectively As in ordinary subjects it was in all cases higher in the sitting than in the supine position (Fig 4) The differences between sitting and supine position are significant (Table II) In relation to pulse rate it varied approximately within the range found for ordinary subjects but was on an average somewhat higher with the heavier work load both in the supine and sitting positions (Fig 9)

The cardiac output at rest averaged 9 18 l/min (range 8 3— 10 0) in the supine and 6 61 l/min (range 5 7—8 3) in the sitting position At rest it showed the same relation to oxygen uptake as in ordinary subjects (BEVEGARD *et al* 1960) in both body positions During exercise it increased parallel with the regression line between cardiac output and oxygen uptake calculated from our earlier normal subjects (Fig 5) At 1 600 kpm/min the cardiac output reached average

Table II. Data obtained in connection with heart catheterization in 8 well trained athletes

Case no	Position	Work load kpm/min	Heart rate beat/min	Ventilation l BTPS/min	Oxygen uptake ml/min	Mechanical efficiency per cent	O_2 capacity ml/100 ml	O_2-sat., per cent		Lactate mEq/l	AV O_2 diff. ml/l
								BA	PA		
1	R	rest	60	7 7	290	—	17.8	99	[illegible]8	1.3	40
	S	rest	68	9 6	374	—	19[illegible]	99	[illegible]9	2.2	61
	S	800	117	40 1	1 [illegible]9[illegible]	25	18 7	99	39	1.2	115
	S	1 600	169	8[illegible] 8	3 1[illegible]0	2[illegible]	19 4	93	6	4 3	144
	R	rest	64	10 1	310	—	16 7	93	78	2.[illegible]	[illegible]
	P	809	119	41 0	1 [illegible]11	2[illegible]	17.8	9[illegible]	4[illegible]	1.[illegible]	94
	R	1 600	1[illegible]	[illegible]2.3	3[illegible]9	2[illegible]	19 1	9[illegible]	[illegible]	6 5	1[illegible]4
2	R	rest	[illegible]	7 7	—	—	17 9	9[illegible]	74	[illegible] 9	45
	S	rest	63	9 4	—	—	18 0	100	63	2.1	68
	S	800	10[illegible]	[illegible]0 1	—	—	18 9	97	40	1 7	109
	S	1 600	1[illegible]1	64.3	9[illegible]	[illegible]4	19 4	93	25	3 9	133
	R	rest	[illegible]	9 4	3[illegible]4	—	17[illegible]	97	74	2.[illegible]	43
	P	8[illegible]0	10[illegible]	[illegible]4 7	1 6[illegible]3	7	18.[illegible]	9[illegible]	43	1.8	100
	P	1[illegible]	149	[illegible]1[illegible]	429	4	19[illegible]	[illegible]3	[illegible]8	5.3	1[illegible]8
[illegible]	P	rest	61	12.4	—	—	20 9	9[illegible]	8[illegible]	[illegible] 9	[illegible]1
	S	rest	[illegible]1	—	—	—	—	—	—	—	—
	S	800	10[illegible]	41.2	1,[illegible]44	[illegible]4	[illegible]1[illegible]	97	4[illegible]	1 4	114
	S	1 [illegible]00	1[illegible]0	8[illegible]1	3 [illegible]84	24	[illegible]19	97	31	1.8	14[illegible]
	P	rest	[illegible]6	12.[illegible]	[illegible]3	—	20 4	97	84	2.0	27
	I	8[illegible]0	109	[illegible]9 8	1 [illegible]33	[illegible]	20 9	9[illegible]	[illegible]4	1.3	94
	P	1 600	1[illegible]	9[illegible]	3 37	[illegible]4	[illegible]19	[illegible]	[illegible]8	3 8	13[illegible]
[illegible]	R	rest	64	10 9	[illegible]	—	18.[illegible]	99	80	[illegible]8	[illegible]8
	S	rest	68	11	[illegible]89	—	1[illegible]	99	67	[illegible]3	63
	S	800	112	40 9	1,914	23	[illegible]0 0	97	43	1 9	111
	S	1,600	1[illegible]4	[illegible]1.8	3 417	[illegible]4	[illegible]0 6	97	[illegible]9	[illegible]7	143
	P	rest	61	9 8		—	18 5	98	[illegible]6	1 9	[illegible]4
	P	800	111	[illegible]8.	1 [illegible]	[illegible]	19[illegible]	97	4[illegible]	1.[illegible]	9[illegible]
	P	1 600	1[illegible]	[illegible]81	[illegible]9[illegible]	[illegible]5	[illegible]0.3	9[illegible]	31	3 9	134
[illegible]	R	rest	8[illegible]		3[illegible]	—	18.[illegible]	97	81	[illegible] 9	[illegible]
	R	800	1[illegible]4	3[illegible]	1,[illegible]	23	18.8	94	4[illegible]	2.3	93
	P	1,600	1[illegible]4	[illegible]9 4	[illegible]01		1[illegible]8	[illegible]	[illegible]0	6.5	1[illegible]0
	P	rest	[illegible]8	9[illegible]	413	—	18.2	95	[illegible]9	3.5	31
	S	800	1[illegible]	[illegible]2	1,8[illegible]3	[illegible]3	19 4	94	[illegible]8	2.[illegible]	11[illegible]
	S	1 600	1[illegible]	[illegible]1 0	3,[illegible]	2[illegible]	[illegible]0[illegible]	9[illegible]	[illegible]	4[illegible]	144

Cardiac output l/min	Stroke volume ml	Pressures mm Hg									
		RV			PA			PCV	BA		
		S	D	De	S	D	M	M	S	D	M
7 3	12	20	— 1	6	19	8	13	—	1?7	80	98
6 1	90	?2	2	5	18	9	13	—	1?3	87	100
15 6	134	43	0	5	31	12	21	—	150	84	116
?1 9	130	67	0	6	40	12	23	—	19	90	1 3
8 6	134	23	0	6	18	9	13	7	128	74	93
18 1	15?	33	0	6	30	9	20	11	161	77	106
26 7	152	57	— 2	4	50	16	29	20	20?	93	136
—	—	23	1	7	23	10	15	—	119	72	85
—	—	21	2	5	15	8	12	—	141	8?	97
—	—	38	4	9	3	14	3	—	187	8?	109
25	169	49	0	7	40	1	?6	—	240	8?	119
8 7	156	22	1	6	2?	8	15	10	116	67	81
16 8	160	40	1	8	40	18	30	22	143	66	??
?6 9	180	55	— 4	3	47	13	32	20	196	80	116
10 6	173	23	— 1	3	16	6	10	—	113	66	85
—	—	18	— 1	2	18	9	11	—	107	73	—
16 2	158	43	0	5	3?	10	19	—	160	87	115
23 3	155	60	— 2	5	45	9	24	—	182	85	11
1 4	193	28	—	2	20	7	13	6	145	78	103
18 4	169	39	— 3		?9	6	17	12	167	74	98
5 6	16?	67	—10	—5	40	7	22	16	219	85	121
10	161	33	1	9	8	12	19	—	130	69	94
6 3	93	24	2	4	0	10	14	—	148	93	115
17 ?	154	40	5	9	30	15	24	—	181	9	1?4
4 0	155	46	3	7	39	14	26	—	202	89	130
8 3	156	3	— 1	5	21	7	15	12	13?	74	97
17 5	158	42	— 2	7	38	14	9	21	164	77	111
24 6	158	61	— 3	9	49	15	3	—	186	94	134
11 9	14	33	— 1	7	26	10	18	1	112	66	90
21 5	173	41	— 1	5	38	13	25	16	149	6	101
27 7	169	54	— 4	6	5?	10	29	16	179	80	113
13 5	159	23	— 1	2	21	2	12	—	110	68	83
16 9	1 8	45	— 3	3	35	17	22	—	166	83	107
25 5	14?	6?	— 5	5	49	13	27	—	174	77	10?

Table IV (cont)

Case no	Position	Work load kpm/min	Pulse rate beats/min	Ventilation l BTPS/min	Oxygen uptake ml/min	Mechanical efficiency per cent	O_2 capacity ml/100 ml	O_2 sat per cent BrA	O_2 sat per cent PA	Lactate meq/l	AV O_2 diff ml/l
6	R	rest	62	8 2	332	—	16 4	98	77	2 3	37
	S	rest	61	9 0	351	—	17 0	97	67	1 3	53
	S	800	106	36 1	1 858	24	18 2	97	38	1 6	109
	S	1 600	149	77 0	3,295	25	18 5	97	27	3 6	132
	R	rest	64	9 3	371	—	16 8	98	76	1 6	39
	R	800	112	35 6	1 751	26	17 4	97	4[illegible]	1.2	90
	R	1 600	155	87 0	3 328	25	18 5	95	30	4 8	123
7	R	rest	57	8 7	336	—	18 8	98	75	1 4	46
	S	rest	68	11 6	424	—	19 2	98	73	1 1	51
	S	800	120	32 9	2 001	22	19 7	94	42	1 1	105
	S	1 600	161	59 5	3 504	24	20 0	91	26	3 0	13
	R	rest	71	9 7	373	—	18 3	100	80	1 9	37
	R	800	121	35 2	1 863	24	19 7	96	45	1 5	103
	R	1 600	160	69 7	3 495	24	20 8	92	30	4 0	130
8	R	rest	62	7 6	311	—	19 5	97	82	1 6	30
	S	rest	58	12 4	384	—	19 6	9	62	1 6	68
	S	800	108	33 0	1 758	25	20 1	94	41	1 8	108
	S	1 600	162	68 8	3,281	25	20	92	27	3 0	134
	R	rest	66	9 3	326	—	18 5	96	79	1 7	34
	R	800	119	31 8	1 650	27	19 1	94	51	1 5	84
	R	1 600	165	74 5	3 0[illegible]8	27	20 6	92	3	4 9	12[illegible]

R = recumbent S = sitting BA = brachial artery PA = pulmonary artery

values of 26 26 l/min (range 24 6—27 7) in the supine and 24 50 l/min (range 21 9—26 5) in the sitting position and these values fell around the extrapolated regression lines for the ordinary subjects in both body positions The cardiac output was always *significantly less in the sitting than in the supine position* both at rest and during exercise (Table II)

The stroke volum at rest during the first determination in the supine position was 149 ml (range 122—173) and during the second determination was 151 ml (range 134—193) The difference is not significant

On changing from supine to sitting position the stroke volume decreased by an average of 43 ml a decrease of 29 % This decrease is of the same absolute

C rd a utput l/m n	Str ke volume ml	Pressures, mm Hg									
		RV			PA			PCV	B A		
		S	D	De	S	D	M	M	S	D	M
9 0	145	26	— 4	5	20	6	12	—	90	56	67
6 7	109	25	0	3	27	11	15	—	121	77	93
17 0	160	44	— 1	3	40	11	23	—	173	77	103
25 0	167	64	—	0	46	10	25	—	19	81	114
9.5	148	26	— 3	2	20	3	10	7	116	60	77
19 5	174	45	— 6	5	39	13	27	16	160	67	96
27 2	175	67	— 7	7	44	5	23	11	225	88	126
7 4	129	23	2	9	2	11	16	—	140	74	99
8 3	123	25	3	7	25	9	15	—	1 6	9	120
19 1	159	46	4	14	43	22	32	—	30	83	1 2
26 5	16	64	0	1	49	25	37	—	250	113	151
10 0	140	7	4	10	23	12	16	12	115	64	83
18 2	150	47	4	13	41	18	30	20	194	87	124
26 9	168	59	7	11	54	24	38	25	65	85	14
10 5	1 0	25	— 1	8	24	8	15	—	114	66	83
5 7	98	20	— 1	2	16	8	11	—	107	67	3
16 2	150	32	— 3	4	22	11	17	—	157	68	93
24 4	151	73	— 3	6	62	10	33	—	18	79	101
9 6	146		— 2	5	20	7	13	9	112	61	80
19 6	16	35	— 4	1	33	8	20	9	161	69	9
24 6	149	53	— 5	7	40	9	4	11	217	77	107

RV = right ntricl PCV = pulmonary cap ll ry venous S = systol c D = in tial-diastol De = end d a s l D = d s l c M = mean

order as in o dinary subjects (BEVEGÅRD *et al* 1960) The difference in stroke volume between the second determination in the supine position and the deter mination in the sitting position was 38 ml or 27 % of the value in supine posi tion (Table II)

In the supine position the stroke volume increased on transition from rest to exercise by an average of 13 ml or 9 % to an average of 163 ml This increase is significant ($p < 0\ 001$) There was no further change in the stroke volume at the heavier load (Fig 6) The coefficient of variation calculated from the dif ferences in stroke volume between the two work loads was 7 4 % in the supine and 5 0 in the sitting position These values are of the same order as the

standard error of a single determination for the stroke volume during work calculated from duplicate determinations (HOLMGREN and PERNOW 1960)

In the sitting position on transition from rest to exercise the stroke volume increased by 49 ml or 48 % of the value at rest After this initial increase the stroke volume on the average remained constant at the heavier load but on a slightly lower level than during work in the supine position (Fig 9) At the first work load it was 14 ml and on the second 9 ml smaller in the sitting than in the supine position (Table II) The difference was probably significant at the second load The postural changes in stroke volume were of the same absolute order as in ordinary men (BEVEGÅRD *et al* 1960)

The stroke volume at rest in the supine position averaged 2 01 % of the blood volume the same as found in ordinary subjects (BEVEGÅRD *et al* 1960 HOLMGREN *et al* 1960 a) The stroke volume in supine position divided by the blood volume was within the normal variation for ordinary young men both at rest and during work (Fig 6)

Intracardiac and intravascular pressures

The mean values and standard deviations are presented in Table III and the individual values in Table IV In the supine position the systolic pressure in the brachial artery rose from an average of 123 mm Hg at rest to 211 mm Hg at 1 600 kpm/min In the sitting position the corresponding values were 132 and 202 mm Hg From 86 observations in ordinary subjects (HOLMGREN *et al* 1960 a BEVEGÅRD *et al* 1960) the mean pressure in the brachial artery in relation to the cardiac index was found to follow the regression line shown in Fig 7 In the present group of athletes the mean pressure in the brachial artery appeared to follow the same regression line in most cases At the highest load however case no 1 4 and 7 had a higher mean pressure in the brachial artery than expected from this regression line *i e* they had a higher resistance in the systemic circulation As a consequence of the larger stroke volumes the pulse amplitude in the brachial artery during exercise was considerably larger in the athletes than in the ordinary individuals (Fig 8) The pulmonary arterial wedge pressure was measured only in the supine position It increased from an average of 9 4 mm Hg (range 6—12) at rest to 15 9 (range 9—22) and 17 0 mm Hg (range 11—25) at the first and second work loads respectively This increase is more marked than in the ordinary subjects (Fig 8) The pressure in the pulmonary artery at rest was within the same range as in ordinary subjects During exercise the mean pressure in the pulmonary artery was higher in the athletes in proportion to the higher pulmonary arterial wedge pressure The pressure gradient over the pulmonary vascular bed in relation to cardiac index followed approximately the same regression line as found for ordinary subjects (Fig 7) In case no 3 however the decrease in pulmonary vascular resistance during exercise was more marked The systolic pressure in the right

ventricle varied within the same range as in the ordinary subjects at rest and during work of corresponding intensity. In the supine position it increased from 24 mm Hg at rest to 58 mm Hg at a work load of 1 600 kpm/min. In the sitting position the values were 22 and 61 mm Hg respectively. As often seen in normal subjects a pressure gradient was recorded over the pulmonary valve during exercise as a result of the high velocity of flow (JONSSON 1957). At rest in the supine position the end diastolic pressure of the right ventricle was equal in the athletes and in the ordinary subjects. At rest in the sitting position it was higher in the athletes than in the nonathletes. The difference is probably significant ($p < 0.02$). It did not decrease during exercise as found earlier in ordinary subjects (BEVEGÅRD *et al.* 1960). In the sitting position it increased slightly. During work it was higher in the athletes than in the ordinary subjects both in the sitting and supine positions. The differences are significant ($p < 0.01$) in the sitting and probably significant ($p < 0.05$) in the supine position.

Discussion

Correlations between size and function of the cardiovascular system in subjects adapted to severe physical work have been studied by several authors (ÅSTRAND 1952, SJÖSTRAND 1953 a, HOLMGREN 1956, REINDELL *et al.* 1957, ROSKAMM *et al.* 1961). Athletes have a large total amount of hemoglobin, a large blood volume in relation to body weight and a large heart volume proportional to the large total amount of hemoglobin (KJELLBERG, RUDHE and SJÖSTRAND 1949, SJÖSTRAND 1953 b, 1955, HOLMGREN 1956). The oxygen transport capacity expressed as the maximum oxygen uptake (*cf.* ÅSTRAND 1952), maximum oxygen pulse (*cf.* ROSKAMM *et al.* 1961) or the rate of work performed at a heart rate of 170 beats/min (PWC_{170}) (SJÖSTRAND 1947, WAHLUND 1948) is greater than in nonathletes. The degree of anaerobic work, expressed as the lactate concentration in arterial blood, is lower than in nonathletes during exercise with the same heart rate (HOLMGREN 1956, HOLMGREN and STRÖM 1959). The present series has all these circulatory criteria of well trained athletes.

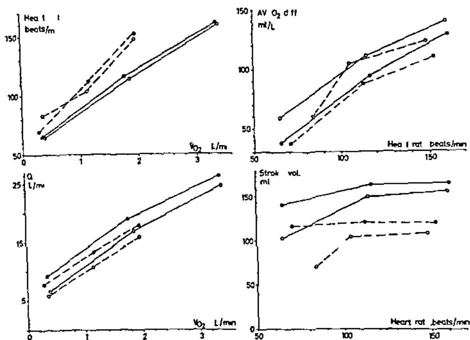
The methods used in this study including the introduction of catheters may alter the circulation. Judging by the influence on heart rate and oxygen uptake this occurred to some extent. Thus the heart rate was 17 % and the oxygen uptake 22 % higher than during basal conditions *prior* to heart catheterization. The influence on the oxygen uptake was slightly more marked than in earlier series of normal subjects during identical conditions (HOLMGREN *et al.* 1960 a, BEVEGÅRD *et al.* 1960). During exercise the influence on the heart rate was negligible, which means that the PWC was approximately the same as before heart catheterization. The procedure was such that two consecutive work tests were performed, separated by a resting period of 30—45 min in

recumbency Thus the first work test may have influenced the second one Before the second exercise started however restitution of pulse rate oxygen consumption and cardiac output had occurred Also during heart catheterization the PWC_{170} was on the average equal at the two determinations

In cases no 2 7 and 8 the arterial oxygen saturation decreased slightly during exercise at the highest work load in both sitting and supine positions This decrease appeared to be caused by hypoventilation which might have been induced by the breathing valve (*cf* HOLMGREN and LINDERHOLM 1958) although its resistance was very low The lactate concentration in arterial blood in these cases was not different from that observed in the other athletes nor were there any significant differences in pressures resistance in the systemic and pulmonary circulation or mechanical efficiency It was therefore thought justified to treat these cases together with the other athletes

Adaptation of the circulatory system to heavy aerobic exercise implies an increase of the oxygen transport capacity This can occur through an increase of stroke volume peripheral oxygen utilization (A V oxygen difference) or both of these factors MUSSHOFF *et al* (1959) compared athletes and ordinary subjects studied in the supine position They found the A V oxygen difference to be larger in athletes The average difference between the two groups was 10 ml per liter blood at rest and during slight work if values at corresponding pulse rates are compared The difference was larger during heavy work amounting to 30 ml per liter blood at a pulse rate close to 170 beats/min During work the stroke volume was 15—30 ml larger in the athletic group At rest however it was the same in the two groups With a lower pulse rate at rest the athletes had a cardiac output one liter per min lower with an oxygen uptake slightly higher than the ordinary individuals The stroke volume increased during exercise in both groups but to a greater extent in the athletes Thus according to MUSSHOFF *et al* (1959) both a larger stroke volume and higher oxygen utilization explained the high oxygen transport capacity of their athletes

The main difference between our athletes and the nonathletes earlier studied (BEVEGARD *et al* 1960 HOLMGREN *et al* 1960 a) is the size of the stroke volume The higher oxygen transport capacity in the athletes can be explained by a larger stroke volume The A V oxygen difference is only slightly higher (about 10 ml per liter blood) in relation to heart rate They did not increase the stroke volume during exercise in the supine position to the extent described by MUSSHOFF *et al* (1959) On transition from rest to moderate work it increased by 9 % and then remained constant during work of higher intensity with an average pulse rate of 160 beats/min (Fig 9) This variation of the stroke volume in the supine position is of the same relative order as in our ordinary individuals and in agreement with what FREEDMAN *et al* (1955) reported They described three athletes whose stroke volume during work with a pulse rate of 141 beats/min was 13 % above the resting value The increase in cardiac output as exercise increases is therefore mainly a result of an increase in pulse rate This is



F g 9 M an alu f the v bles ın th F k equ t on at est and d ıng e c s ın p (filled les) nd tt g po t n (open cles) F ll l p esent the p esent gr up f ll t ed thletes (val es n T bl II) and b oken l es an li gr p of non athl tes stud d ın th m m n (BE EGÅRD *et al* 19 0)

ın agreement with the results reported by WANG *et al* (1960 c) who investigated three athletes

The cardiac output and the oxygen uptake at rest were larger in the athletes than in nonathletes (Fig 5) The relationship between cardiac output and oxygen uptake at rest was about the same in both groups *i e* the A V oxygen difference was about equal During work of moderate intensity the cardiac output for a given oxygen uptake was somewhat larger in the athletes but during heavy work it was the same as expected from the regression line for ordinary subjects The deviation from this regression line at rest and at the first work load may be due to the fact that the athletes had a larger oxygen uptake at rest during catheterization (when compared to the predicted basal value) than the nonathletes The effect of body position on the cardiac output and stroke volume (Fig 5 6 and 9) was of the same absolute order as in ordinary subjects In relation to the larger stroke volume and blood volume the absolute decrease in stroke volume on transition from supine to sitting position was less important in the athletes This may explain the less marked orthostatic pulse reaction in the athletes

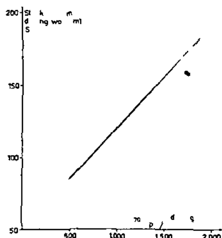


Fig 10

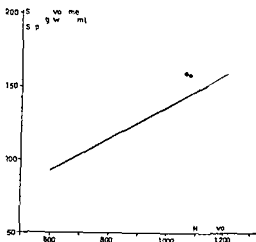


Fig 11

Fig 10 Stroke volume (SV) during work in supine position (mean values of both determinations) in relation to work intensity at a pulse rate of 170 beats/min (PWC) during heart catheterization in supine position The line indicates the regression calculated from earlier investigations (HOLMGREN *et al* 1960 a BEVEGÅRD *et al* 1960) Thin broken lines represent $\pm$ one standard error of estimate Regression equation SV = 49 1 + 0 0716 PCW r = 0 88 S D = $\pm$ 9 5 n = 7

Fig 11 Stroke volume (SV) during work in supine position (mean values of both determinations) in relation to heart volume (HV) Symbols as in Fig 10 Regression equation SV = 25 9 + 0 111 HV r = 0 83 S D = $\pm$ 11 1 n = 27

ercise in the sitting position caused an increase of the stroke volume as in ordinary individuals The mechanism seems to be a redistribution of blood from the lower part of the body to the central veins with subsequent increase of ventricular filling (BEVEGÅRD *et al* 1960) During continued work of higher intensity the stroke volume remained constant however and was about 10 ml smaller than in the supine position This difference in stroke volume between the two body positions is compensated for by a higher A V oxygen difference in the sitting position so that the oxygen transport per pulse beat is equal Therefore the PWC_{170} was independent of the body position

In normal subjects close correlations have been found between the size and function of the cardiovascular system (HOLMGREN *et al* 1960 a) This is corroborated by the present results as the difference in oxygen transport capacity between the ordinary subjects and the athletes is mainly due to the size of the stroke volume The relationship between the stroke volume and the physical working capacity at a pulse rate of 170 beats/min follows approximately the same regression in both groups (Fig 10) The slightly higher physical working capacity in the athletes than predicted from the regression found for ordinary subjects corresponds to a slightly larger arterio-venous oxygen difference in

the athletes at a heart rate close to 170 beats/min (Fig 9) In relation to the heart volume the stroke volume in the athletes is somewhat larger than expected from the regression obtained in ordinary subjects (Fig 11) A large cardiovascular system is a prerequisite for the high functional capacity in athletes Our group of athletes had a higher working capacity than expected from their stroke volume and a somewhat larger stroke volume than would be expected from the size of the heart These deviations might be due to the fact that these athletes were a selected group of the best among competition cyclists They show a more efficient adaptation to muscular work than other subjects with the same circulatory dimensions HOLMGREN *et al* (1960 b) have shown that after short term intensive training ordinary subjects increase the functional capacity of the circulation more than they increase their heart volume and blood volume

During heavy exercise with a heart rate close to 160 beats/min the intracardiac and intravascular pressures are considerably higher in the athletes than in the ordinary subjects (Fig 8) In relation to the cardiac output however the pressure levels were of the same order except for the ventricular filling pressures which were higher in the athletes Concerning work intensity oxygen uptake and cardiac output the first work load performed by the athletes corresponded approximately to the second load performed by the ordinary subjects There was no difference in the vascular resistances of the pulmonary and systemic circulations in the two groups (Fig 7)

The high pulmonary arterial wedge pressure during heavy exercise in athletes is probably a result of a large central blood volume This may represent a higher potential energy which may help in the maintenance of a large stroke volume with a short effective filling time at high heart rates (SJOSTRAND 1953 a)

In the athletes there was a less marked decrease of right ventricular filling pressure after change of body position from supine to sitting This corresponds to a relatively smaller change in stroke volume If the initial-diastolic pressure in the right ventricle is assumed to be a measure of the intrathoracic pressure the difference between end-diastolic and initial-diastolic pressures should represent the effective filling pressure As it is difficult to determine these pressures during heavy exercise the figures are only approximative (Table III) The values obtained imply that the effective filling pressure is lower in the sitting than in the supine position at rest This increases during work more in the sitting than in the supin position Thus the stroke volume varies with the filling pressure indicating that Starling s law may operate during the circumstances studied here In the supine position however there are usually optimal filling conditions for the ventricles and a further rise in filling pressure will give a less marked increase of the stroke volume as noted by RUSHMER, SMITH and FRANKLIN (1959)

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Some Effects of Vitamin D and Parathyroid Hormone on the Calcium and Phosphorus Metabolism of Bone in Vitro

By

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Rece ed 14 J n 1962

Abstract

NICHOLS G Jr S SCHARTUM and G M VAES *S me ffcts f vitam n D a d pa athyroid hormone o the c l um and pho phorus met bolism f bone in vit* Acta physiol scand 1963 *57* 51—60 — The nfluen e of Vitam n D on serum calcium le els cannot be expla ned by the effect on intestinal absorption of calcium alone Rather some alt ration in the distribut on of calcium between bone and the circulat ng fluids must occu a concept that has rec ved in d rect experimental suppo t In the study reported belo bone (cal aria from mice) was incub ted *i vitro* until a steady state equ l brium for alcium and phosphorus between the bone and the incubat ng flu d was reached thus at the end of the incubation s mul ting the tuation *n vivo* The conc ntrat ons of these ions that bon from animals pre t eated with Vitamin D maintained n the urrounding flu ds were s gnif cantly h ghe th n V tam n D deficient c ntrols demonstrating d rectly the calcium mobiliz ng effect of th itam n Further V tamin D increased the passive olub lity of th bone m neral and lactate p oduction by bone cells suggest g modif cations of bone cell m tabolism In the absence of V tamin D parathyro d hormone had the same effects as eported for th tam ind at ng that the funct on of parathyroid hormon is i d pendent of the p esen e of Vitamin D Some ev dence is presented that in m c depr ed of Vitam n D secondary hyper parath roidism exists

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It is now well established that Vitamin D enhances intestinal absorption of calcium Yet as pointed out by NEUMAN and NEUMAN (1958 p 156) the elevation of serum calcium concentration seen in hypervitaminosis D and its depression in Vitamin D deficiency when accompanied by hypoparathyroidism cannot be explained on this basis alone Rather some alteration in the distribution of calcium between bone and the circulating fluids must occur as a result of the action of this vitamin

This concept has received indirect experimental support from several laboratories CARLSSON (1952 1954) demonstrated that Vitamin D raised serum calcium concentrations in the rat even on a diet practically devoid of calcium By manipulating the calcium content of a low calcium rachitogenic diet NICOLAYSEN and EEG LARSEN (1956) showed that Vitamin D treated rats exhibited higher serum calcium levels than did Vitamin D deficient rats despite the fact that the deficient animals absorbed more calcium from the intestine than the Vitamin D treated animals From these results it was inferred that the increased amounts of calcium in the serum of the Vitamin D treated animals could come only from the skeleton In addition LINDQUIST (1952) found that the administration of Vitamin D did not increase the specific activity of the serum calcium despite an increase in the amount of radiocalcium entering the blood from the intestine suggesting that Vitamin D simultaneously increased the amounts of non radioactive calcium entering the d stream from the bones

In the study reported below samples of bone were incubated *in vitro* until a steady state equilibrium for calcium and phosphorus between the bone mineral and the incubating fluid was reached thus at the end of the incubation simulating the situation *in vivo* (SCHARTUM and NICHOLS 1961) The concentrations of these ions that bones from animals pre treated with Vitamin D were able to maintain in their surrounding fluids were significantly higher than controls demonstrating directly that Vitamin D has an effect on the skeleton This effect together with some aspects of the possible underlying mechanism and the interrelationship between the function of Vitamin D and parathyroid hormone are discussed below

Methods

Pre treatment of the animals

Male white Swiss mice from the age of 4 weeks were fed Rachitogenic Diet No 2 U S P (Nutritional Biochem cals Corporation) consisting of

Ground Gluten	20
Ground Whole Yellow Maize	76 /
Calcium carbonate	3 /
Sodium chloride	1

Since this d et is very poor in phosphorus as well as lacking Vitamin D 4 NaH_2PO_4 was added Having been on this diet for 3—4 weeks (during wh ich the animals appeared

healthy) some of the animals were injected subcutaneously with a single dose Vitamin D[1] 3 days prior to sacrifice In some experiments the dose was 8 000 U in others 70 U When parathyroid hormone was given the animals were injected subcutaneously with 10 U (Injection Parathyroid U S P 100 U S P units per ml) daily for 3 successive days the last dose being given 18 hours prior to sacrifice At this age the animals weighed 30—40 g For each experiment bones from experimental mice were incubated and compared with Vitamin D deficient controls from the same litter

Prepa ation f tis e samples

The animals were killed by cervical fracture and decap tated immediat ly In some experiments blood was collected for serum analysis following decapitation The calvaria were removed and the bones freed from tendinous and muscular attachments by dissection and adhe ent blood and clots were remo ed by washing the samples in ice cold Krebs-Ringer b carbonate medium All bones w re kept in fresh media stored on ice until the start of the incubat on After gentle blotting two calvaria were pooled (about 100 mg total weight) weighed and placed in the incubating flask

Heat inacti at n

When bone samples were killed by heat ng the procedure was as follows Samples of glucose free medium w e heated to boiling emoved from the gas flame and the bone samples quickly added and kept in the hot media for 1 1/2 min They were then transferr d to f eshly p epared media for incubation In a previous communication (SCHARTUM and NICHOLS 1961) evid nce as given that this heating procedure does not s gnificantly ch nge mineral olubility or rates of calcium and phosphorus dif fusion

Incub tio

The samples were incubated for 6 hours n a Dubnoff metabolic incubator at 37 8 C n 2 ml of mod fied Krebs-Ringer medi buffered to pH 7 4 with HCO_3^- and 5 / CO_2 Penicillin at a final concentration of 5 U per ml and Streptomycin 0 01 mg pe ml we e added to prevent bacterial growth Glucose as used as substrate at a oncentrat on of 2 mg per ml During the incubation a continuous stream of 95 / O_2 with 5 CO was fed nto the hood o ering the flasks whi h were left in the incubator ithout stoppers All media were also equilibr ted for 10 min with the gas mixture b fo e being pl ced in the flasks In such a syste n at the end of 6 hours incubat on the calcium and phosphorus conc ntrations measured in the media can be cons de ed to be n a steady state equilibr um with the bone mineral and thus simulate th situation *in* *t o* as has been d scussed elsewhere (SCHARTUM and NICHOLS 1961)

Criteri n f b ne c ll vi b lity

Prev ous experienc ind cated that bone samples ncubated under the present con ditions ar v able after 16 hours incubation. On th other hand cellular metabolism c ases completely n bon samples heated as described

A lytical te hn ques

Aliquots of the media were analyzed fo calcium by the EDTA-calcium chelating method with murexide as n ind cator (MUNSON *et* *l* 1955) for phosphorus by the m thod of FISKE and SUBBAROW (1925) and for lactate by a modific tion of BARKER and SUMMERSON s techn que (1941)

[1] De 3 Hydros 1 Wander

Th m dia contained n calcium Ph sphorus was present at a oncentration of 0 40 mm l /l

Table I Pre treatment with 8 000 U vitamin D Fresh bone

	Ca conc.	P co c.	Ca × P conc.	Lact. conc
Vitamin D deficient				
Mean	100	100	100	100
No	10	10	10	7
S D	2 3	2 6	2 0	7 6
Vitam n D treated				
Mean	113	111	126	1 1
N	9	9	9	
S. D	9 3	16 5	24 0	12 ?
p	<0 001	N S	<0 01	<0 01

The follow ng symbol are used in th nd subsequent tables

Mean values expressed as per nt of valu f r tam n D defic t c t ls.
No of determinat ons.
Stand rd deviat on
Not ignif cant.

Calculatio

Th re was a tendency for minor shifts to occur n the absolute concentrat ons of calcium and phosphorus from one day to another while the relative differences between x perimental samples and controls remained quite constant Therefore all values have calculated as per c nt of controls for each experiment Bessel s correction for small samples was us d in calculat ng standard deviations The significance of the difference between means was estimated using Student s t test

Results

I *Pre treatment with 8 000 U Vitamin D Fresh bone*

In preliminary experiments in which the animals were injected with 800 U Vitamin D daily for 3 days prior to sacrifice no significant increase in medium calcium concentrations was observed Therefore in subsequent ex periments a higher dose was given When 8 000 U was injected (no inter mediate dosage level was tried) as a single dose 3 days prior to sacrifice the concentrations of calcium the calcium phosphorus product and lactate in the media were significantly increased see Table I Phosphorus concentrations rose also but this difference was not significant

In contrast to these effects observed after *in vi o* vitamin administration Vitamin D added *in vitro* in final c ncentrations of 6 000 to 20 000 U per ml of medium was without effect on medium calcium concentration

II *Pre treatment with 20 U Vitamin D and with parathyroid hormone Fresh b ne*

It might be objected that 8 000 U of Vitamin D is a tremendous d se in a mouse producing pharmacological or toxic effects rather than

Table II Pretreatment with 20 U vitamin D and with parathyroid hormone Fresh bone

	Ca conc	P conc	Ca × P conc
Vitamin D deficient (PTE treated)			
Mean	100	100	100
No	9	10	9
S D	4.0	4.9	6.7
Vitamin D treated (PTE treated)			
Mean	108	108	120
No	10	10	10
S D	4.4	4.9	6.3
P	<0.001	<0.01	<0.001

physiological ones However since a much smaller dose produced no significant effects in preliminary experiments special experimental conditions were designed in an attempt to demonstrate the effect using a dose of 20 U which could be considered in the physiological range

The normal concentration of serum calcium and low serum phosphorus usually observed in human rickets and osteomalacia has been attributed to parathyroid hyperactivity brought on by the feedback mechanisms for control of calcium ion concentration postulated by McLEAN 1958 — a concept which is supported by the finding of parathyroid enlargement (FOURMAN 1960 p 132) In the present experiments Vitamin D deficient mice exhibited normal serum calcium concentrations (in contrast to the hypocalcemia often found in young rachitic rats) suggesting that the parathyroids were functioning normally Since in previous experiments (SCHARTUM and NICHOLS 1961) treatment with parathyroid extract increased the steady-state calcium concentrations maintained in the incubation media the possibility existed that hyperparathyroidism secondary to Vitamin D deficiency was present in the experimental D deficient mice which masked the effects of physiological doses of the vitamin on medium calcium concentration

In order to eliminate the effect of possible secondary hyperparathyroidism parathyroid hormone (PTE) was given to both groups In our experience the dose used produces a pronounced hyperparathyroidism and it was felt that any hyperactivity of the glands would then cease completely Under these circumstances the hormone effect would be equal in both groups and the exclusive effect of the vitamin could then be studied The results are presented in Table II As can be seen 20 U of the vitamin resulted in a significant increase in calcium phosphorus and the calcium × phosphorus concentration products in the media Lactate production was not determined in these experiments

Table III Pre treatment with 8 000 U vitamin D and no parathyroid hormone Heat inactivated bones

	Ca conc.	P conc.	Ca × P co c
Vitamin D deficient			
Mean	100	100	100
No	8	8	8
S D	3 9	3 6	4.5
Vitamin D treated			
Mean	110	112	124
No	8	8	8
S D	5 9	9 6	12 9
P	<0 01	<0 01	<0 001

III *Pre treatment with 8 000 U Vitamin D and no parathyroid hormone Heat in activated bone*

In a previous communication it was shown (SCHARTUM and NICHOLS 1961) that the calcium and calcium × phosphorus concentration products that surviving bone samples were able to maintain in their surrounding media were higher than those maintained by bone samples in which cellular activity had been destroyed by heat However although bones from animals pre treated with PTE maintained higher calcium × phosphorus concentration products than controls this effect was also seen when heat inactivated bone samples were used From these experiments it was concluded that parathyroid hormone increases the passive solubility of bone mineral In the present work therefore it was appropriate to investigate whether this phenomenon could also be demonstrated for the vitamin Bone from Vitamin D treated animals was heated and incubated together with heated controls As can be seen from Table III bones from vitamin treated animals maintained significantly higher concentrations of calcium phosphorus and calcium × phosphorus products than controls Thus it would appear that the vitamin like the hormone in creases the passive solubility of bone mineral

IV *Pre treatment with parathyroid horm ne and no Vitamin D Fresh bone*

It has been shown (HARRISON HARRISON and PARK 1958) that parathyroid hormone is ineffective in rats deprived of Vitamin D This may explain why D deficient rats usually exhibit low serum calcium and normal serum phosphorus levels indicating a lack of effective compensatory hyperparathyroidism However the usual findings of normal serum calcium and low serum phosphorus levels in human osteomalacia and rickets suggest that in man adequate hormone function is independent of the presence of the vitamin The results presented in Table IV and V clearly show that in D deficient mice parathyroid hormone significantly elevates serum calcium levels and calcium phosphorus and

Table II Pre-treatment w h parathyroid horm n. and no v tam n D Fresh bone

	Ca onc.	P conc.	Ca × P conc.
Vitamin D deficient			
Mean	100	100	100
N	4	4	4
S. D	4.8	.5	.8
Vitamin D deficient (PTE treated)			
Mean	11?	110	1 3
No	4	4	4
S D	3 8	5 0	7 7
p	<0 02	<0 0?	<0 01

Table I Effects on serum Ca concentrations of treatment with pa athyr id hormone in v tamin D defic ency

	Ca (mMol/l)		Ca (mM l/l)
Controls		PTE-treated	
1	/6	1	3.91
2	2 65	2	3.56
3	?.82	3	3.35
4	41	4	3.50
Mean	? 66	Mean	3.58
S D	0 18	S D	0.–4

Th difference betw een th means is gnificant at better than the 99% confidence level.

calcium × phosphorus concentration products in the media as well Thus it would appear that in mice hormone function is independent of the presence of the vitamin [1] Therefore the general conclusion drawn by HARRISON and HARRISON (1960 p 302) that Vitamin D is required for the action of the parathyroid hormone should be restricted to the rat the experimental animal used

Discussion

Since in the experiments reported here the media initially contained no calcium all calcium measured in the media at the end of the incubation was released from the bone samples Bones from animals pre-treated with the vitamin released significantly more calcium than controls This may be taken as direct evidence of a calcium mobilizing effect on the skeleton of the vitamin

[1] Since this work was finished we ha been informed f the demonstration in the adult rat of an increase in th serum calcium co centration up to and beyond the normal range fter administrat on of moderat doses of parathyroid extract to parathyroid-ectomized rats which had been fed itamin D-free diet fo 8 months. (Toverud S U personal communi cat on)

The mechanism for the vitamin effect is not clear However it may be useful to compare the results obtained for living and dead bones As can be seen from Table I and III the increase in the concentrations of calcium phosphorus and calcium × phosphorus product was about the same in these two types of experiments From this observation the conclusion can be drawn that Vitamin D increases the calcium × phosphorus product concentration at least partly by increasing the passive solubility of the mineral In this respect the effect is similar to that of parathyroid hormone

Another prominent feature of the action of parathyroid hormone upon bone cellular metabolism is an enhancement of aerobic lactate production This has been shown consistently in this laboratory (BORLE NICHOLS and NICHOLS 1960 SCHARTUM and NICHOLS 1961 VAES and NICHOLS 1962) and has been confirmed by others (NEUMAN and DOWSE 1961 RAISZ AL and TEPPERMAN 1961) The data in Table I indicate that in this respect also the action of Vitamin D resembles that of parathyroid hormone The amount of lactate produced was 21 higher in the vitamin treated group but the per cent increase might have been higher had not secondary hyperparathyroidism been present in the vitamin deficient group It is of interest to note that the enhanced lactate production by bones from animals pre treated with the vitamin is opposite to what might have been predicted on the basis of Neuman s compiled data from studies on cartilage From these he suggests that lactate (and pyruvate) produced by bone cells should accumulate in Vitamin D deficiency and the production of citrate should be reduced (NEUMAN 1958 p 162) Conversely Vitamin D should favor citrate formation from lactate and pyruvate The present results do not support this view In addition although it is well established that Vitamin D increases citrate concentrations in bone and serum HARRISON *et al* (1958) found that there was no necessary correlation of Vitamin D effect on calcium metabolism and citrate levels

The rationale for using the medium calcium and phosphorus steady state concentrations in experiments with heat inactivated bone to estimate the passive (thermodynamic) solubility of the bone mineral has been discussed at length (SCHARTUM and NICHOLS 1961) Similarly the possible relationship of the increased rate of lactate production to the changes in mineral solubility and the higher levels of calcium and phosphorus found in the incubation media surrounding bone from animals treated with parathyroid hormone has been reviewed extensively in recent publications from this and other laboratories (BORLE *et al* 1960 NEUMAN and DOWSE 1961 SCHARTUM and NICHOLS 1961 RAISZ *et al* 1961 VAES and NICHOLS 1962) Therefore a rehearsal of these arguments would contribute little to this communication Rather it seems important to re-emphasize that on the basis of the experiments outlined above Vitamin D appears to modify the metabolism of bone fragments *in vitro* and that these effects are very similar to those observed following treatment with parathyroid hormone Indeed it seems likely (cf Table II and

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The Effect of Cardioacceleration by Methylscopolamine Nitrate on the Circulation at Rest and During Exercise in Supine Position, with Special Reference to the Stroke Volume[1]

By

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Abstract

BEVEGARD S *The effect of cardioacceleration by methylscopolaminenitrate on the circulation at rest and during exercise in supine position with special reference to the stroke volume* Acta physiol scand 1963 57 61—80 — In 9 healthy adult males the work intensity at pulse rate 170 (PWC_{170}) was determined before and after i. m. injection of 0 75 mg methylscopolaminenitrate (MSN) The PWC_{170} decreased 14 after MSN In 15 patients with normal circulation the effect of MSN was studied with heart catheterization at rest and during work at two progressive loads in the supine position At rest the heart rate increased 66 the stroke volume decreased 34 and the cardiac output was unchanged The ventricular filling pressures decreased. During work, performed after MSN ventricular filling pressures and stroke volume increased progressively At the highest work load the stroke volume was only 12 lower than before MSN Oxygen uptake cardiac output, central blood volume and vascular resistances in the systemic and pulmonary circulations were unchanged after MSN both at rest and during work. After MSN the heart volume, determined in the prone position tended to decrease. The duration of the mechanical diastole as measured from the phonocardiogram, was significantly shorter after MSN both at rest and during exercise. The investigation elucidates the relationship between the size of the stroke volume and the capacity for work at a given pulse rate and also between the size of the stroke volume and ventricular filling pressure

[1] Preliminary results were reported at the annual meeting of the Swedish Medical Society in 1959 and 1960

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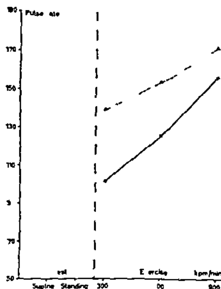


Fig 1 Pulse rate at rest in the supine and standing positions and during exercise in the sitting position before (circles and full line) and after MSN (triangles and broken line) Mean values obtained from 9 healthy male subjects Average age = 26 years (range 20—35)

artery The blood from the sampling site passed through a cuvette densitometer (XC-100 A Waters Co) at a speed kept constant at about 20 ml/min by the aid of a motor driven syringe The concentration time curves and a signal for the dye injection were recorded on a Speedomax recorder The cardiac outputs and central blood volumes were calculated according to the Stewart Hamilton equations (STEWART 1897 HAMILTON *et al* 1932)

Results

The effect of MSN on the pulse rate at rest and during exercise

The pulse rate at rest in supine position increased by 77 % from an average of 60 beats/min to 106 beats/min after injection of MSN After 8 minutes standing the pulse rate was on an average 83 beats/min before and 134 beats/min after MSN corresponding to an increase from the values in supine posture of 38 % and 26 % respectively There was no significant difference concerning the increase in pulse rate from supine to standing position before and after MSN Also during exercise the pulse rate was higher after MSN but the difference decreased gradually with increasing work load (Fig 1) The rate of work performed at a pulse rate of 170 beats/min (PWC_{170}) was on the average 1 069 kpm/min before and 914 kpm/min after MSN corresponding to a decrease of the PWC_{170} by 14 % from the control value This decrease is significant ($p < 0.01$) There was an approximately linear relationship between pulse rate and work load both before and after MSN and it appears from Fig 1 that the two lines tend to converge at a pulse rate of 190 beats/min which is near the expected level of the maximal pulse rate for this age group (ROBINSON 1938 ÅSTRAND 1952)

Table I Some anthropom t ic data of 15 patients with normal card ac function

C se n	Sex	Age y rs	H ght cm	W ght, kg	B S A m	He rt v lum ml	Total hemo-gl bin g	Bl od olume l	Work inten ty kpm/ m n
A 1	♂	31	183	66 6	1 89	8 0	701	4 9	980
A 2	o	14	165	48 3	1 53	600	5?0	4 1	700
A 3	♂	?8	183	90.2	2 13	1 140	1 004	6 3	1 630
B 4	♀	0	16?	73..	1 0	590	673	5.3	560
B 5	♀	18	16?	56 3	1 60	560	556	3 7	710
B 6	o	39	177	7? 5	1 90	—	?9?	5 9	730
B 7	♂	14	15?	58 6	1 56	560	457	3.3	630
B 8	o	16	171	61 9	1 74	570	79?	5 5	9?0
B 9	♀	33	167	67 0	1 75	640	49?	4 0	7?0
B 10	♀	23	166	71.5	1 80	610	6?8	4 9	720
C 11	o	21	173	70 5	1 85	8 5	715	4 8	700
C 12	o	15	174	57 3	1 71	680	568	5 1	900
C 13	♀	25	167	61 9	1 70	580	442	4 3	600
C 14	♂	19	177	78 4	1 96	880	919	6 0	1 50
C 15	♂	41	168	53 3	1 61	760	6?7	5.2	800

At a puls t of 170 b ts/min in tting pos t
N t in ste dy state

The effect of MSN on the central circulation

Data of the subjects investigated with heart catheterization (Table I) The hemoglobin concentration which on the average amounted to 14 1 g per 100 ml blood (range 12 5—16 1) for the male subjects and 12 5 g per 100 ml blood (range 10 6—13 3) for the female subjects was within normal range in all cases except for C 13 where it was slightly low

The heart volume in relation to the total hemoglobin (THb) was within two standard deviations from the normal regression line (HOLMGREN *et al* 1957) in all cases except for B 8 where it was smaller In case B 6 no value for the heart volume was obtained The physical working capacity at a pulse rate of 170 beats/min (PWC) in relation to the THb was within two standard deviations from the normal regression line (HOLMGREN *et al* 1957) in all cases except for B 4 and B 6 where it was slightly lower In relation to the heart volume the PWC was also within this range in all cases except for C 11 where it was slightly lower

Data obtained during heart catheteri ation (Table II)

The pulse rate at rest before MSN and during the first determination was 81 beats/min (range 60—114) This was on the average 36 , higher than during basal conditions prior to heart catheterization In 6 cases a second

Table II Data obtained in connection with right heart catheterization of 15 patients with normal

Case no Cath. no MSN Dose mg	B = before MSN A = after MSN	Work load kpm/min	Pulse rate beats/min	Oxygen uptake ml/min	Mechanical efficiency per cent	O_2 capacity ml/100 ml	O_2 sat. per cent Br A	PA	AV O_2 diff ml/l
A 1	B	rest	60	257	—	19 3	98	81	36
148/59	A	rest	110	254	—	19 1	99	83	3?
0 75	A	450	133	1 132	24	20 1	96	56	81
	A	900	154	1 796	28	20 7	96	41	116
A ?	B	rest	95	248	—	16 8	97	83	?7
151/59	A	rest	139	237	—	17.2	97	8?	27
0 50	A	300	1[illegible]	804	?4	17 [illegible]	98	61	66
A 3	B	e t	65	55	—	19 9	98	80	37
168/59	A	rest	99	264	—	19 8	99	83	33
0 75	A	600	131	1 489	24	19 9	96	55	83
	A	1 200	154	2 699	?4	19 9	96	42	110
B 4	B	est	106	236	—	17 1	98	82	29
62/60	B	250	128	867	18	17 5	98	6	66
0 75	B	500	159	1 240	23	17 7	98	53	84
	B	re t	102	248	—	16 7	99	83	8
	A	r st	15?	260	—	16 7	99	83	28
	A	?50	171	817	20	17 6	97	59	68
	A	500	191	1,269	23	17 7	97	51	83
B 5	B	re t	78	245	—	17 8	99	81	35
76/60	B	300	120	879	21	18 9	98	60	74
0 50	B	600	164	1 304	?6	19 1	96	53	84
	B	rest	96	274	—	17 7	98	8?	29
	A	rest	142	279	—	17 6	98	83	?8
	A	300	159	872	21	18 2	96	[illegible]	63
B 6	B	re t	86	56	—	17 3	99	8[illegible]	25
95/60	B	200	95	760	18	17 6	100	63	67
0 75	B	400	106	989	25	17 8	96	53	?8
	A	r st	122	292	—	17 2	98	84	27
	A	200	142	735	18	17 7	97	67	54
	A	400	158	1 143	21	18 1	98	62	68
B 7	B	r t	114	2?6	—	18 3	98	81	31
119/60	B	50	144	849	19	18 6	96	63	63
0 50	B	500	170	1 368	21	19 0	94	53	?9
	B	r t	113	30	—	18.2	96	85	22
	A	rest	157	?9?	—	17 9	99	8[illegible]	26
	A	50	177	878	18	18 0	97	63	65
	A	500	190	1 364	21	18 5	100	49	95

MSN = methyl copolam ne itrat RV = r ght v nt le PA = pulm nary t ry PCV =
D = diastolic M = me n.

ti ulat n

Ca d output l/m n	Str ke volum ml	Pressures mm Hg								
		RV		PA			PCV	B A		
		S	De	S	D	M	M	S	D	M
2	120	20	1	19	5	12	7	E11	58	78
7 9	71	16	−3	13	5	9	4	121	75	9?
13 9	105	24	−1	?3	10	15	11	160	81	103
15 5	101	—	—	6	13	19	11	—	—	—
9 3	97	22	3	17	8	12	7	99	63	8?
8 8	63	21	1	16	8	11	—	105	2	8
12 1	75	31	1	?3	11	14	—	118	6	87
6 9	106	25	4	18	8	11	4	116	68	85
7 9	80	19	0	14	4	8	0	1 1	80	94
17 9	137	3(	0	21	10	14	5	169	3	99
24.5	159	48	1	36	8	17	4	168	90	116
8 3	78	15	2	15	5	9	5	10	69	80
13.2	103	25	0	23	8	12	5	109	/0	87
14 9	93	28	1	22	7	1	6	125	67	89
8 9	87	16	2	14	5	9	—	97	69	8
9 3	61	19	0	14	6	10	3	94	68	77
12 0	/0	20	1	18	9	14	5	111	72	9
15..	80	26	1	2?	8	16	—	1?6	73	96
7 0	90	23	6	16	8	11	—	127	57	90
11 9	99	38	3	33	15	24	—	157	7	107
15 6	9	47	5	38	2?	31	—	0?	91	123
9 5	99	23	3	15	9	1?	—	137	7?	92
10 1	1	2	1	15	9	1	—	137	77	10?
13 8	87	36	0	30	17	23	—	164	84	110
10 3	120	26	5	5	8	15	8	1 4	67	90
11 3	119	39	7	33	1?	21	12	1 6	87	119
12 7	120	5	4	—	—	19	—	188	91	118
10 8	88	30	3	2..	10	15	6	126	71	9?
13 6	9	46	5	36	13	23	10	164	86	113
16 8	106	58	9	—	—	24	—	1 0	8?	110
9 5	84	30	3	22	8	13		140	84	108
13 4	93	33	2	28	11	16	—	169	8	11
17.2	101	37		36	9	19	—	184	75	11?
14.2	125	28		17	6	11	—	145	72	102
11 3	2	27	1	25	8	11	—	13	7	96
13 (		40	1	37	10	21	—	160	77	106
14 4	6	45	1	43	12	3	—	177	67	107

p lm n ry c p ll ry venous B A = bra hial artery S = systol c D = end-dia t l

Table II (cont.)

Case no. Cath no MSN Dose mg	B = before MSN A = after MSN	Work load kpm/min	Pulse rate beats/min	Oxygen uptake ml/min	Mechanical efficiency per cent	O_2-capacity ml/100 ml	O_2-sat. per cent		AV O_2 diff ml/l
							Br A	PA	
B 8	B	rest	89	352	—	18 6	96	78	34
121/60	B	400	125	1 139	?1	18 9	98	63	68
0 75	B	800	159	1 709	26	19 4	98	5?	9
	B	rest	91	304	—	18 0	98	81	33
	A	rest	136	287	—	18 1	99	83	0
	A	400	161	1 014	25	18 3	?8	60	71
	A	800	174	1 730	26	19 3	96	50	9?
B 9	B	rest	107	273	—	17 0	93	85	4
145/60	B	?50	140	861	16	17 6	97	—	—
0 75	B	500	164	1,261	23	18 1	98	58	75
	A	rest	136	258	—	16.8	98	84	26
	A	250	147	877	18	17 6	98	62	66
	A	500	158	1 348	?1	18 0	97	50	87
B 10	B	rest	65	206	—	16 9	95	80	?8
163/60	B	250	122	797	21	18 1	97	58	71
0 5	B	500	153	1,217	24	18 5	96	49	90
	A	rest	144	220	—	16 9	98	80	31
	A	?50	161	810	20	17 5	96	53	6
	A	500	175	1,260	?3	18 1	96	47	90
C 11	B	rest	63	249	—	13 8	97	79	7
189/60	B	600	155	1 490	23	20 5	97	48	103
0 75	A	rest	158	?88	—	19 7	97	81	35
	A	600	184	1 406	25	20 6	97	49	10?
C 12	B	rest	6	242	—	18 5	97	81	3
149/61	B	700	135	1 457	27	18 4	98	4?	105
0 75	B	rest	81	226	—	17 3	100	80	35
	A	rest	139	27	—	17 6	99	81	34
	A	700	158	1,53?	26	18 4	100	39	115
C 13	B	rest	81	2?1	—	14 2	99	78	1
157/61	B	400	12	99?	4	14 6	99	51	73
0 75	A	rest	147	?40	—	14 1	93	7	30
	A	400	175	923	7	14 9	99	49	78
C 14	B	rest	68	313	—	21 6	99	80	44
159/61	B	900	127	1 931	26	22.0	9	47	109
0 75	A	rest	111	346	—	?0 3	100	83	37
	A	900	150	1 959	25	21 7	96	51	99
C 15	B	rest	75	234	—	16 6	96	78	32
170/61	B	600	1?6	1 330	25	16 5	97	46	86
0 75	B	rest	8	216	—	15 6	98	79	33
	A	rest	117	2?7	—	15 7	99	84	?
	A	600	148	1 345	?5	16 9	97	45	89

Card. output, l/min	Stroke volume, ml	Pressures, mm Hg								
		RV		PA			PCV	BA		
		S	De	S	D	M	M	S	D	M
10 4	116	29	4	24	8	15	9	103	57	74
16 7	134	38	4	29	12	21	12	139	68	97
18 6	117	51	4	37	13	24	—	171	82	108
9 3	107	24	3	17	7	12	—	107	55	70
9 5	70	23	7	—	—	—	—	107	65	75
14 2	88	41	2	34	12	21	9	119	65	8
18 9	103	5	4	4	14	25	—	161	80	103
11 6	108	30	3	24	6	14	7	154	7	108
—	—	33	2	27	5	14	10	155	64	112
16 8	10	37	7	27	5	14	—	186	80	118
9 8	2	19	0	18	4	9	3	147	88	106
13 4	91	29	1	25	9	15	7	163	83	113
15 6	98	36	2	24	5	15	—	177	80	117
7 4	114	28	5	25	9	15	—	118	0	97
11.2	97	34	1	31	10	16	10	147	72	96
13 5	88	38		30	8	17	—	159	73	101
7 1	49	26	0	22	6	13	—	118	86	93
10 6	66	40	1	32	10	17	8	127	75	91
13 9	80	51	2	47	10	15	—	149	75	101
6 7	106	23	4	17	4	10	7	120	71	9
14 4	93	46	0	39	10	20	6	170	84	112
8 3	53	25	0	21	4	12	5	133	94	108
13 7	74	47	6	36	11	20	9	189	9	118
7 5	120	26	6	26	10	17	6	108	0	87
13 8	103	4	1	31	1	20	—	164	6	107
6 5	80	18	3	17	6	11	—	108	65	87
6 6	48	19	1	18	5	13	—	107	6	79
13 4	8	4	9	37	9	23	—	04	72	105
7.2	88	18	2	18	4	9		93	60	77
13 6	107	37	0	29	6	13	—	127	71	9
8 1	55	2	0	19	5	12	—	10	6	8
11.8	68	4	0	25	6	14	—	129	4	93
7 1	104	2	7	2	11	16	—	115	73	87
17 8	140	47	6	29	17	23	—	150	8	108
9 4	85	17	1	10	1	7	—	106	78	93
19 8	13	53	4	33	13	20	—	154	77	103
7.2	96	18	5	18	4	10	6	100	69	83
15 4	127	4	8	31	1	2	—	135	0	98
6 6	85	19	3	15	3	9	—	96	68	83
9 0	77	20	1	17	5	11	—	107	80	93
15 1	10	43	4	29	9	20	—	131	73	9

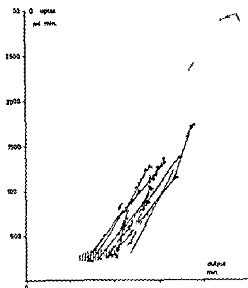


Fig 2 Oxygen uptake in relation to cardiac output at rest (filled symbols) and during exercise before (circles connected by full line) and after MSN (triangles connected by broken line) The area of normal variation obtained from determinations on 27 healthy subjects in the supine position (HOLMGREN *et al* 1960 BEVEGÅRD *et al* 1960) is surrounded by a dotted line The hatched area represents the normal variation at rest

determination at rest before MSN was performed after the first exercise test and the pulse rate was then on the average 7 % higher than during the first determination After MSN the pulse rate was 134 beats/min (range 99—158) corresponding to an increase of 66 % from the control value

The pulse rate during work In group B where two progressive loads were performed the pulse rate at the first load was on the average 125 beats/min (range 95—144) before and 160 beats/min (range 142—177) after MSN With the second load it was 152 beats/min (range 106—170) and 174 beats/min (range 158—191) respectively After MSN the pulse rate was on the average higher by 28 % at the first load and by 15 % at the second load The differences were significant ($p < 0.001$) and probably significant ($p < 0.05$) respectively

The oxygen uptake at rest was 15 % (range —12— + 49) higher than the predicted basal oxygen uptake (HARRIS and BENEDICT 1919) As earlier observed (BEVEGÅRD *et al* 1960) restitution has occurred in about 30 min after exercise at submaximal loads There was no significant difference in oxygen uptake after MSN

The oxygen uptake during work There was no significant difference between the oxygen uptake before and after MSN As the work loads were the same it follows that the mechanical efficiency was the same This averaged at the first work load 20 % and at the second load 23 %

The oxygen saturation of the arterial blood varied within normal limits at rest as well as during work The oxygen capacity of the arterial blood increased during work as in normal subjects (BEVEGÅRD *et al* 1960) Administration of MSN did not cause any significant difference

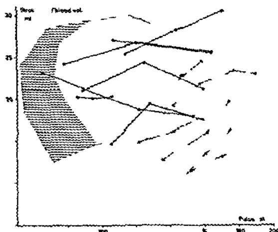


Fig 3 Stroke volume divided by blood volume in relation to pulse rate at rest and during exercise before and after MSN Symbols as in Fig 2

The oxygen saturation of mixed venous blood (Fig 4) at rest was slightly higher after MSN and the difference was probably significant ($p < 0.05$) During exercise however there were no significant differences at the same oxygen uptake

The arterio-venous oxygen difference (Fig 4) at rest was on the average 32 1 ml/l (range 24—44) before and 30 0 ml/l (range 25—37) after MSN the difference being probably significant ($p < 0.05$) During exercise there were no significant differences at the same oxygen uptake

The cardiac output (Fig 2 and 4) at rest before MSN amounted to 8 22 l/min (range 6 7—11 6) and after MSN to 8 92 l/min (range 6 6—11 2) The difference was not significant During work the cardiac output increased with the first load to 13 99 l/min (range 11 2—16 7) before and to 12 88 l/min (range 10 6—14 2) after MSN With the second work load the values were 15 62 l/min (range 12 7—18 6) and 15 79 l/min (range 13 9—18 9) respectively The cardiac output during exercise was not significantly altered after injection of MSN In relation to the oxygen uptake the cardiac output both at rest and during exercise (Fig 2) was within the normal range in most cases In case B 9 the cardiac output was higher than normal at rest but tended to normalize during work In cases B 6 and B 7 it was somewhat high at rest after MSN and in case B 6 this occurred also during work A somewhat high cardiac output was also observed in case B 8 on the first work load

The stroke volume (Fig 3 and 4) before MSN at rest was 103 ml (range 78—120) During work it was 107 ml (range 92—134) at the first load and 104 ml (range 88—120) at the second load These changes are not significant An approximate value of the error of a single determination was obtained by calculation from the variations between the determinations at the two

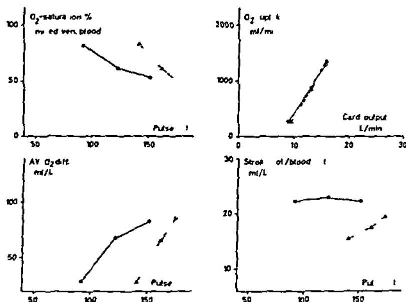


Fig 4 Mean values of some hemodynamic relationships at rest and during exercise before and after MSN Symbols as in Fig 2

work loads and amounted to 5 8 % In relation to the blood volume the stroke volume was within normal limits (BEVEGÅRD *et al* 1960 HOLMGREN *et al* 1960) in most cases (Fig 3) In relation to the THb the stroke volume during work in all cases was within two standard deviations from the normal regression line (BEVEGÅRD HOLMGREN and JONSSON 1962) The relationship between the stroke volume during work and heart volume (BEVEGÅRD *et al* 1962) was normal in most cases In case B 8 the stroke volume was larger and in case C 11 slightly smaller than expected from the value of the heart volume

After MSN the stroke volume at rest showed a significant decrease ($p < 0.001$) which on the average amounted to 36 ml (range 12—72) or 34 % of the control value On transition from rest to exercise the stroke volume increased by 19 ml or 28 % of the value at rest and during work with a heavier load by another 10 ml It was then 44 % higher than at rest after MSN These increases were significant ($p < 0.01$ and $p < 0.02$ respectively) Although the stroke volume increased on transition from rest to exercise and during continued exercise with a heavier load it was significantly lower than before MSN both at the first ($p < 0.01$) and second load ($p < 0.02$) The average difference was at the first load 26 ml or 25 % of the value before MSN and at the second load 12 ml, or 12 % of the value before MSN

The central blood volume (cases C 11—C 15) before MSN and at rest was on the average 886 ml (range 754—1 090) corresponding to 17 4 % (range 16—18) of the total blood volume During work it was 1 167 ml (range 936

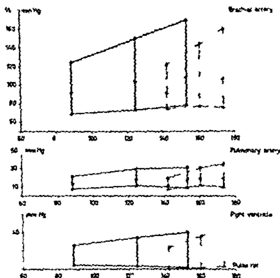
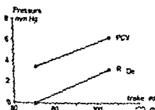


Fig 5 Mean values of pressures from brachial artery pulmonary artery and right ventricle at rest and during exercise before and after MSN Symbols as in Fig 2



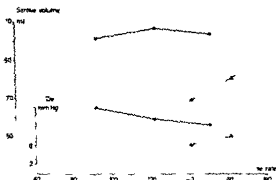


Fig 6 Fig 7

Fig 6 Mean values of pulmonary capillary venous mean pressure (PCV) and right ventricular end-diastolic pressure (RV_{De}) in relation to stroke volume before (filled circles) and after MSN (open circles)

Fig 7 Mean values for stroke volume and right ventricular end-diastolic pressure (RV_{D}) in relation to pulse rate at rest and during exercise before and after MSN Symbols as in Fig 2

—1 400) After MSN the central blood volume was 828 ml (range 735—1 001) at rest and 1 198 ml (range 915—1 450) during work. The differences before and after MSN were not significant The increase from rest to exercise was significant ($p < 0.001$) and amounted to an average of 32 %

The intracardiac and intravascular pressures (Fig 5 6 and 7) The pressure in the brachial artery was on the average normal at rest and during exercise (HOLMGREN *et al* 1960 BEVEGÅRD *et al* 1960) After MSN at rest the pulse

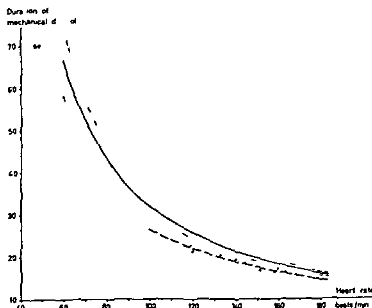


Fig 8 The effect of MSN on th du at on of m chan cal d ast le in r lat on tc h a t rat t rest and during exercise Th normal l t on hip nd cat d by th full li e Th dotted l es epresent ± on tand rd rror of estimat Eq ation y 10⁴/(4 03 — 8 J 2) r — 0 99 n — 7 Th he vy broken lin l uld) p x mat ly f t th observations after MSN

amplitude in the brachial artery decreased depending on a significantly (p < 0 01) higher diastolic pressure The systolic and the mean pressures were not significantly altered after MSN As the cardiac output was the same before and after MSN it follows that the peripheral vascular resistance was unchanged The pressures in the pulmonary artery were normal at rest and during exercise (BEVEGÅRD *et al* 1960 HOLMGREN *et al* 1960) and no significant changes occurred after MSN The pulmonary vascular resistance showed no significant changes after MSN The systolic pressure in the right ventricle which was normal at rest and during work was also essentially unchanged after MSN The pulmonary capillary venous pressure and the end diastolic pressure of the right ventricle were normal at rest and during exercise in all cases where they were recorded As earlier observed (BEVEGÅRD *et al* 1960) the end diastolic pressure of the right ventricle on the average decreased slightly during work Administration of MSN caused significant decreases (p < 0 001) of the pulmonary capillary venous pressure and the end-diastolic pressure of the right ventricle (Fig 6 … pressures after MSN increased during work (Fig 7) The in… end-diastolic pressure with the higl… nificant (p < 0 02) The pulmonary cap… measured only in a few cases but

The effect of MSN on heart volume and duration of mechanical diastole

The heart volume at rest in the prone position showed a tendency to decrease ($p < 0.02$) after MSN. The decrease was on the average 87 ml (range 0—220). In three of the seven cases the decrease amounted to 100 ml or more.

After MSN the mechanical diastole was significantly shorter ($p < 0.001$) in relation to pulse rate at rest and during exercise (Fig. 8).

Discussion

The material used for the present studies of the circulatory adaptation to increased heart rate by administration of methylscopolaminenitrate (MSN) consists of young subjects with normal circulation as far as can be judged by the results of clinical and laboratory examinations and for some of them also by heart catheterization. Each subject was used as its own control. NALEFSKI and BROWN (1950) showed that the effect of atropine on heart rate varies with age and is most pronounced during the first three decades of life.

The methods employed have been the same as earlier reported and discussed (BEVEGÅRD *et al.* 1960). Among the cases investigated with heart catheterization group B and C performed one work test before and one after administration of MSN. After the first exercise test restitution of pulse rate and oxygen uptake had occurred before MSN was given. After MSN the measurements were not commenced until the pulse rate was stable.

Anticholinergic influence on pulse rate and stroke volume at rest

The present investigation has demonstrated that administration of an anticholinergic substance methylscopolaminenitrate (MSN) in doses of 0.50—0.75 mg (8—14 µg per kg body weight) increases the heart rate and decreases the stroke volume so that no significant change of the cardiac output occurs. The cardioaccelerator effect of anticholinergic substances has been well documented (GOODMAN and GILMAN 1956 p. 546). Concerning the effect of atropine in large doses (1—2 mg) on stroke volume and cardiac output varying results have been reported. Differences in vagal tone depending on disparity in age of the materials studied and the time interval between the administration of the drug and the measurements may account for some of the contradictory results. In some studies where the measurements were made within 3 min after i. v. injection of atropine a significant increase of the cardiac output with no (WEISSLER *et al.* 1957) or slight decrease (GORTEN *et al.* 1961) of the stroke volume was found. It may be uncertain whether a circulatory steady state is obtained within this short time interval. From studies by GORLIN (1958) and by WILBER and BRUST (1958) it appears that the heart rate was not stable until 5—15 min after i. v. injection of atropine. GORLIN (1958) who studied the effect 10—15 min after injection in subjects of somewhat higher age found no change in cardiac output. BERRY *et al.* (1959) found significant

increase of the cardiac output within 1—3 min after i. v. injection and compared to this value a slight decrease after 14 min. These immediate and late effects also appear from the investigation by McMichael and Sharpey Schafer (1944). The present investigation where the effect was studied 15—20 min after i. m. injection of MSN when the heart rate was stable, is in agreement with previous reports where it has been found that anticholinergic cardioacceleration results in a decrease of the stroke volume.

A decrease in stroke volume can depend on impaired filling or emptying of the ventricles. As also demonstrated by others (McMichael and Sharpey Schafer 1944, Weissler et al. 1957, Gorlin 1958, Berry et al. 1961) after injection of atropine the filling pressure for both the right and left ventricles decrease. This decrease could be explained by lower intrathoracic pressure, a decrease of venous tone or a redistribution of blood within the vascular system. As anticholinergic substances apparently alter esophageal tone, intraesophageal pressure does not reveal accurately changes in the intrathoracic pressure (Berry et al. 1959). With MSN no changes in respiratory rate or ventilation were observed. It is improbable that any change in the intrathoracic pressure occurred.

The observation in the present study that ventricular filling pressure and stroke volume decrease after MSN is thus consistent with an impaired ventricular filling due to a decrease of the effective filling pressure. The tendency to a decrease of the heart volume after MSN is also consistent with a diminished ventricular filling. However, the results concerning the heart volume may be uncertain because the exposures were not trigged to end diastole. Kjellberg, Rudhe and Sjöstrand (1951) found no change of the heart volume in end diastole after MSN. The hemoglobin concentration was unchanged after MSN, indicating that the total blood volume was not altered. This is in agreement with the results of Gorten et al. (1961). No change in the central blood volume was observed. The same result was obtained by Berry et al. (1959) and by Gorten et al. (1961) after atropine. They also found a decrease of ventricular filling pressure and stroke volume. Weissler et al. (1957) found unchanged stroke volumes and larger central blood volumes after atropine. Gorten et al. (1961) found that the volume in which an indicator was diluted during its first circulation was unchanged after atropine when the indicator was injected into the superior vena cava but decreased when the indicator was injected peripherally into antecubital vein or femoral vein. The results of Gorten et al. (1961) indicate the importance of central injection and sampling for studies of changes in the central blood volume. This is also pointed out by McIntosh et al. (1961), who suggest that changes in arterial volume or in the distribution of flow can change the value of the central blood volume. In the present study injection of indicator and sampling occurred centrally. With the assumption that the indicator dilution technique reflects real changes in the volume of the vascular bed the results obtained in this and in the studies

by BERRY *et al* (1959) and by GORTEN *et al* (1961) should imply a decrease of central venous tone and a decrease of peripheral venous volume On the other hand the current techniques for estimation of the central blood volume may be erroneous during the conditions studied KALSER *et al* (1954) and MILLER *et al* (1954) suggest from their results that i m injection of 2 mg atropine causes peripheral vascular pooling

Studies on the effect of atropine on the peripheral circulation in animals have given varying results After large doses vasodilatation has been observed (HAMET 1936) No direct effect was noted by EULER (1938) on the nerve free vessels of human placenta GASKELL (1956) found no appreciable effect immediate or delayed on the rate of blood flow through the human hand or forearm after i a injection After i v injection of 2 mg atropine HORSLEY and ECKSTEIN (1959) found significant decrease of pressure and volume in human forearm veins and this response was not influenced by inflation of a lower body antigravity suit Vasodilatation of superficial vessels atropine flush is a well known phenomenon and occurs also after MSN It is not known whether by this mechanism alone a sufficient amount of blood could be deverted from the effective circulating volume so as to explain the marked effect on the central circulation According to WOLLHEIM (1927) the subpapillary plexuses of the human skin constitute a reservoir of considerable volume If pooling of blood occurs after anticholinergic cardioacceleration it is therefore possible that the pooled blood is not available in the large veins This hypothesis is supported by the observation that certain maneuvers intended to increase venous flow such as head-down tilting and passive leg raising failed to increase the cardiac output or stroke volume after atropine whereas rapid infusion of serum albumin had a marked effect (BERRY *et al* 1959)

Animal experiments have shown that cardioacceleration with an artificial pacemaker reduces the stroke output (BERGLUND *et al* 1958 RUSHMER 1959 WARNER and TORONTO 1960) In patients with heart block central venous pressure and stroke volume decreased when ventricular rate was augmented by an artificial pacemaker (BEVEGÅRD 1962 a) In patients developing paroxysmal tachycardia a decrease of the stroke volume has also been found (SAUNDERS and ORD 1962 BEVEGÅRD JONSSON and KARLOF 1960) It is possible that at rest an increased heart rate *per se* may influence the distribution of the blood volume and the stroke volume The underlying mechanisms are unknown but it is probable that regulatory adjustments via the pressoreceptors modify the circulatory response to cardioacceleration

Ventricular filling may be restricted by pronounced shortening of the mechanical diastole It appears from Fig 8 that after MSN the duration of the mechanical diastole was shorter than at the same heart rates before MSN It is possible that this more marked shortening of the duration of the mechanical diastole after MSN contributed to the restriction of the ventricular filling volume The average decrease in heart volume corresponded approximately to twice

the decrease in stroke volume The changed relationship between the duration of mechanical systole and diastole after MSN as compared to the state at corresponding heart rates during exercise before MSN may be explained by a difference in myocardial contractility secondary to a difference in sympathetic tone

Anticholinergic influence on pulse rate and stroke volume during exercise

On transition from rest to exercise and during continued exercise with increasing intensity the stroke volume increased gradually and reached almost the same value as before MSN This increase of the stroke volume during work explained the decreasing differences in pulse rate at corresponding loads before and after MSN as the peripheral oxygen utilization was not influenced by MSN After MSN the work intensity at a pulse rate of 170 beats/min was 14 % lower This difference is explained by the difference in stroke volume which amounted to 12 % at the highest work load These observations further demonstrate the close relationship between the size of the stroke volume and the capacity for work at a given pulse rate (cf BEVEGÅRD *et al* 1960 and 196? BEVEGÅRD 1962 b)

During exercise the stroke volume increased parallel to the rise in ventricular filling pressure (Fig 7) This can be explained by increased ventricular filling as a result of a redistribution of blood in a central direction due to the venous pump Usually the filling conditions for the ventricles are optimal in the supine position and a further rise in filling pressure will then have only a slight influence on the stroke volume (RUSHMER SMITH and FRANKLIN 1959)

The increased sympathetic discharge during exercise may adjust the capacity of the venous system and thus adapt the circulation to increasing oxygen transport demands WILBER and BRUST (1958) found that if cardioacceleration was produced by injection of atropine during infusion of norepinephrine the stroke volume decreased only insignificantly and the cardiac output increased considerably RUSHMER (1958) could in dogs by a combination of induced tachycardia and simultaneous administration of epinephrine and norepinephrine more closely reproduce a circulatory response similar to that during exercise than with either of these factors alone

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From the Institute of Neurophysiology University of Copenhagen Denmark

Conditioned Avoidance Behavior of Cats with Lesions in Globus Pallidus

By

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Abstract

LAURSEN A M *Conditioned avoidance behavior of cats with lesions in globus pallidus* Acta physiol scand 1963 57 81—89 — After both unilateral and bilateral lesions in globus pallidus conditioned avoidance responses were extinguished more rapidly than in normal and control operated cats This was observed at a time when retention had returned to normal Animals with bilateral lesions in globus pallidus tested 30 days after the operation learned the avoidance response slower than normal and control operated cats Retention tested 30 days after bilateral lesions in globus pallidus was impaired as compared with normal and control operated cats Sixty days postoperatively retention was normal Therefore a memory defect cannot account for the rapid extinction observed more than 60 days postop at which it may be explained by diminished fear

Experimental studies indicate that learning (ROSVOLD and DELGADO 1956 ROSVOLD MISHKIN and SZWARCBART 1958 DEAN and DAVIS 1959 BATTIG ROSVOLD and MISHKIN 1960) rather than basic motor mechanisms (WILSON 1914 RANSON and BERRY 1941 KENNARD 1944 LAURSEN 1955 1962 a b) are functions of corpus striatum Conditioned avoidance behavior was affected by combined caudate and cortical lesions not by cortical lesions alone (THOMPSON 1959) I placed lesions in the projection areas of the caudate Nucleus niger and globus pallidus (VONEIDA 1959 SZABO 1962) Lesions in nucleus niger were abandoned because they damaged adjacent structures and resulted in generalized muscular weakness Although lesions in globus pallidus included the lateral part of the genu of the internal capsule motor signs did not appear The purpose of the study presented in this report was to investigate the effect of lesions in globus pallidus on conditioned avoidance behavior

Tabl I Fxperimental groups

		Number of c ts
1	B l teral les ons in globus pallidus	12
2	Un lateral les ons in globus pall d s	3
3	B lateral lesions in thalamus	10
4	Sham operated o bilateral les or s in internal capsule	7
5	Unoperat d	14

Method

Material

Sixty seven tame cats (2 6 to 6 kg male and female) were used Successful experiments were made on 46 animals Some cats were discarded because the lesions were misplaced (3) or because they aquired an avoidance response not amenable to quantitative analysis (lay on their backs or balanced on the hurdle 10 operated and 8 unoperated cats)

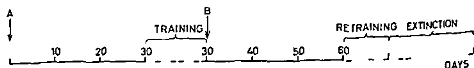
The experimental groups are listed in Table I Lesions were made either before (A Fig 1) or after (B Fig 1) training the number of animals in these subgroups is indicated in Fig 4

Operation

A monopolar electrode insulated except at the tip was introduced stereotactically into the brain under nembutal anesthesia and aseptic conditions Unilateral or bilateral lesions in the globus pallidus or b lateral lesions in the thalamus or internal capsule were made by passing a direct current of 2 to 3 mA for one m nute from the tip of the electrode to a metal plug in the rectum The angle of entry of the electrode was varied so that effects due to the electrode tracts could be detected Three cats had bilateral and two un lateral sham operations The electrode was introduced into globus pallidus but electrolytic lesions were not made After lesions in the globus pallidus the cats were apathetic for about 2 weeks One cat trained 5 days after the operation reached criterion of training in 30 days Therefore avoidance conditioning was started 30 days after the operation After thalamic lesions recovery was rapid Two cats retra ned 5 days after the operation reached criterion in 2 and 3 days

Histology

All lesions were verified histologically After the experiments the brains were perfused and fixed in 10 per cent Formalin Frozen sections were cut at 20 microns every tenth section was stain d with thionine The lesions were pear shaped and occupied a volume of about 5 mm^3 Thus a lesion that destroyed the globus pallidus damaged



F g 1 S quence of the p ocedur In gro p 1 and 3 (Table I) les ns were mad e ther at A or at B All lesion in g up 2 nd 4 wer made at A

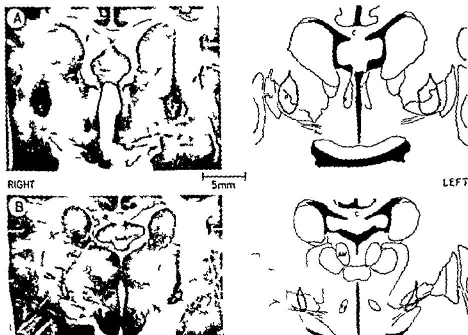


Fig 2 A Example f b lateral les ns n gl bu pall dus th dama to ab nternal apsul (t 87)

B Les ns nterrupt ng th ff nt fibers of gl bu pall dus (t 108)

Th n n tained bra n se on (20 μ) ( )

rresponding d gr m / ghs

Aa Ar amygdal d a ant
AM Nu leus anter or m dialis
AL Ansa lent cul is
CC Corpus callosum
Cd Nu leus aud tus
CI Capsul int rna
Ch Chiasm opt cum
Cl Cl ustrum
En Nu leus ent peduncularis
F F rn x
GP Gl bus pall dus
H Hypo h l mus anteri
HL Hypothal mus l ral s.
P t Putamen
R N leu cula is
RE Nucl us eun ens
TO T c us p cus
VA N cl us n ralis n

the internal capsul as well (Fig 2 A) In one cat mall b lat ral lesions (2 mm) s tuated n th nucleus entopeduncularis nd ansa lent cularis had the same eff ct as les ons destroy ng globus pall dus probably because the ff rent f bers of globus pall dus w re interrupted (F g 2 B

The ommissura ant or passes through the ostrobasal part of globus p ll dus in three of 12 cats the commissura anterior was damaged without aff ting the results

Th h m logue in carni es f h medial segment of gl bus pall dus n primates

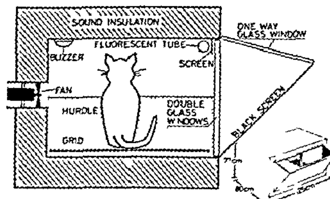


Fig 3 Shuttl box. The one way glass window is tilted to prevent the cat from seeing it own reflection

Avoidance conditioning

Shuttle box The box is shown in Fig 3 The electric buzzer in the roof of each compartment deli ered 50 clicks/sec and the two buzzers were used simultaneously Shocks were applied through floor grids of one or the other compartment from a variable autotransformer (110 V 50/sec A. C) The current drawn from this constant voltage source was calculated from the voltage drop over a 100 Ω resistor in series with the cat this voltage drop was measured with a cathode ray oscilloscope Shock intensities were between 2 and 4 mA A constant current generator as source of shocks (3 MΩ output impedance) as unsuitable because the intensity of the shock was increased when the cat lifted one or two paws A further disadvantage was that the shock was diminished when urine or sal a shunted the cat

Conditioning The response studied was crossing the hurdle that is when two front paws touched the grid floor on the other side On the first day of training the animal was placed in the box for an hour without stimuli Under these conditions the cats crossed the hurdle between 0 and 26 times On the second day only the buzzers were presented for 10 sec at arying intervals if the cat crossed the hurdle the buzzers were stopped Most cats did not cross the hurdle at all others crossed it 1 to 6 times On the third day a grid shock was applied 10 sec after the onset of the buzzers The stimuli were presented 20 times daily at random intervals of 0 to 90 sec The average interval was 60 sec If the cat responded by crossing buzzers and shock were stopped If as occasionally happened the cat neither responded to buzzers nor shock both were stopped 60 sec after the buzzers began

Criterion of training was avoidance of the shock in at least 18 of the 20 daily trials on two successive days This criterion had the disadvantage that the number of trials to criterion was a multiple of 20 On the other hand it was not influenced by irrelevant habits of individual cats some cats made most errors in the beginning others in the middle and others at the end of a session *The criterion of retraining* was the same as the criterion of training To extinguish the conditioned avoidance response the buzzers were presented without the shock, at the same intervals as during training *The criterion of extinction* was absence of hurdle crossing on two successive days during 5 min with the buzzers continuously on Having failed once to respond for 5 min nearly all cats also failed to respond on the next day

Avoidance behaviour was characterized by

a) The number of trials to criterion of training
b) The number of trials to criterion of retraining
c) The number of trials to criterion of extinction

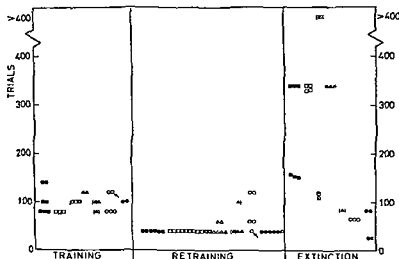


Fig 4 Number of tr als to cr t ri n of train ng retraining and e tinct on in d dual cats
T ai ing Bilateral les ons n globus pallidus delayed acquis tion of the a o da ce response (●) as c mpa ed with 26 unope ted cats
R t g R tent on was del yed after bil teral les ons in globus pall dus mad 30 d ys (○) or 60 d ys bef e (●)
E tinction B lateral d un lateral les ons in gl bus pall dus prod ced rap d extinction (○ ● ◐)
■ Unoperated 30 d ys between tra ing nd retraining
□ Unope ated 6 d y betw en t ining d t ining
Γ Sham perated
◘ B l t ral les on n th intern l psule before training
△ B lateral les ons in th thal mus m de after t ing
▲ *Bil t l les ons in th thal mus m de bef tr ning*
(▲) B l ter l th l m c les ns ext nd g into th fundus of th caud t
○ B l teral les ons in th gl bus p ll dus af er tra ing
○ Cat 108 w th les on h n F g 2 B
● B l teral les o in globus p ll dus bef training
◐ Unil t ral les ns gl bus p ll dus.

d) The avo dance latency d fined as the time from the onset of the buzzers to the a oidance response
e) The escape latency d fined as the time from onset of the shock to an escape response
f) The number of hurdle cross ngs not p eceded by stimuli

Results

1 *General behavior* All 12 cats with bilateral lesions in globus pallidus be came sluggish They did not eat and hardly moved the first 4 or 5 days after the operation The slow recovery was not an unspecific effect of the surgical intervention because 10 cats with bilateral *thalamic lesions* behaved normally two days after the operation Operated cats were indistinguishable from un operated cats with respect to stretch and flexion reflexes muscle tone and pos-

An in est gation of m tabol changes after les ns in gl bus pallidus is n progress.

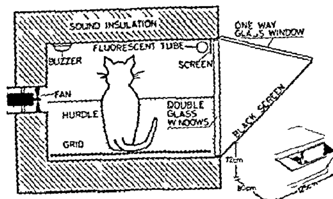


Fig 3 Shuttl box The n way glass wind w i tted to p event the cat from see ng its own r fl ction.

Avoidance conditioning

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Avoidance beha ior was characterized by

a) The number of trials to criterion of training
b) The number of trials to criterion of retraining
c) The number of trials to criterion of extinct on.

Table II Avoidance latencies before (6 cats) and after bilateral lesions in globus pallidus (6 cats) and in unoperated cats (8 cats)

Latency in seconds with standard error of the mean

Operated after training	Training	3.5±0.2	3.4±0.2	3.7±0.3	4.7±0.3	5.1±0.3	7.0±0.3		
	Retraining	5.9±0.4	5.3±0.2	5.0±0.4	7.6±0.3	4.1±0.4	6.7±0.3		
Operated before training	Training	6.7±0.4	4.8±0.3	5.7±0.3	7.6±0.3	5.9±0.4	6.4±0.3		
	Retraining	5.7±0.3	6.3±0.4	5.0±0.3	6.8±0.4	5.0±0.3	5.5±0.3		
Unoperated	Training	5.7±0.3	5.8±0.3	2.7±0.3	3.5±0.3	5.6±0.3	5.7±0.4	5.2±0.4	4.8±0.3
	Retraining	4.1±0.3	3.4±0.2	2.3±0.1	4.4±0.2	4.2±0.3	4.2±0.3	4.7±0.4	5±0.3

tention days (p 4) was 4.9 ± 0.3 sec (14 cats 36—40 trials each) Cats with bilateral lesions in globus pallidus responded with a 25 per cent longer latency (6 cats 36—40 trials each Table II)

During *retraining* 6 of 8 normal cats responded with shorter latencies than during training (Table II) Four of six cats with bilateral lesions in globus pallidus made after training responded with longer latency during retraining In cats with bilateral lesion in globus pallidus made before training the avoidance latencies during retraining were not systematically changed (in one cat it was longer in two shorter in three unchanged) Thus bilateral lesions in globus pallidus increased the avoidance latency and abolished the effect of retraining on avoidance latency

Avoidance latencies in 10 cats with bilateral lesions in the thalamus in 3 cats with unilateral lesions in globus pallidus and in 2 cats with bilateral lesions in the internal capsule did not deviate systematically from avoidance latencies in unoperated cats The slow response of cats with lesions in globus pallidus was not due to a motor handicap The mean escape latency was 2.4 ± 0.2 sec (6 cats 525 trials) in operated cats 2.3 ± 0.2 sec (14 cats 628 trials) in unoperated cats

Discussion

The main result of this study was that conditioned avoidance responses were extinguished faster in cats with lesions in globus pallidus than in normal and control operated cats This was observed at a time after the operation when retention of avoidance responses had returned to normal WEISKRANTZ (1956) and WATERHOUSE (1957) attributed rapid extinction of avoidance responses to diminished anxiety and fear The same interpretation is consistent with the observation in this study that cats with lesions in globus pallidus did not show

signs of fear during transfer from the cage to the experimental box normal and control operated cats vocalized struggled to escape defecated etc

Rapid *extinction* of avoidance responses has been found after combined lesions of the cortex and the caudate nucleus (cats THOMPSON 1959) after large basotemporal lesions (cats BRADY *et al* 1954) after lesions of the amygdala (monkeys WEISKRANTZ 1956) after allo-juxtallocortical lesions (monkeys PRIBRAM and WEISKRANTZ 1957) and after prefrontal lobotomy (monkeys WATERHOUSE 1957)

In contrast to THOMPSON (1959) who reported normal *acquisition* of an avoidance response when training began 15 days after combined cortical and caudate lesions acquisition was delayed when tested 30 days after lesions were placed in globus pallidus This discrepancy may not be due to the different sites of the lesions because THOMPSON stated that high variability might have masked an effect on acquisition Slow acquisition of avoidance responses was found two weeks after basotemporal lesions in cats (BRADY *et al* 1954) one week after amygdala lesions in monkeys (WEISKRANTZ 1956) and one week after allo-juxtallocortical or frontal isocortical lesions in monkeys (PRIBRAM and WEISKRANTZ 1957)

In cats with lesions in globus pallidus *retention* of the avoidance response was impaired temporarily only Retraining was delayed 30 but not 60 days after the operation After basotemporal (BRADY *et al* 1954) and amygdala lesions (WEISKRANTZ 1956) retention was unaffected one and two weeks postoperatively although the amygdala hangover (WEISKRANTZ 1956) seems to indicate a slow recovery similar to that observed after lesions in the globus pallidus A marked loss in retention was found two weeks after prefrontal lobotomy in monkeys (WATERHOUSE 1957) and one week after damage to the interpeduncular nucleus in rats (THOMPSON 1960) Maintenance of avoidance responses without exteroceptive stimuli (SIDMAN 1953) was affected one week after lesions of orbital insular and temporal cortex in monkeys as well as after lesions of the amygdala (WEISKRANTZ and WILSON 1958)

My results may be explained by diminished fear of a stimulus warning of a shock the slow postoperative recovery may be due to a temporary metabolic defect That retention of avoidance responses had returned to normal while fear still seemed to be diminished supports BRADY *et al* s (1954) suggestion that retention is not dependent on fear That retention was found normal may however reflect only the experimental design more sensitive measures of retention might have shown a lasting defect The results can be interpreted without assuming that learning or memory in general were deranged nor do they exclude that globus pallidus has other than emotional functions

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Über den initialen Anstieg der O_2-Kapazität des Blutes der Ratte bei Hypoxie

von

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Abstract

TRIBUKAIT B *Über den initialen Anstieg der O_2 Kapazität des Blutes der Ratte bei Hypoxie* Acta physiol scand 1963 *57* 90—98 — Rats were submitted to hypoxia corresponding to 6 000 m altitude The effect on hemoglobin concentration hematocrite plasma protein concentration total hemoglobin and the specific activity of the erythrocytes tagged in vivo with C^{14} 2 glycine has been studied during the first two days The hemoglobin and plasma protein concentrations increased significantly They were significantly correlated to each other There is no indication that the spleen should act as a blood reservoir during these circumstances The specific activity of hemin was unchanged which indicates that no essential hemolysis or increase of the circulating hemoglobin occurred However the total hemoglobin increased significantly Thus the amount of hemoglobin in the erythropoietically active tissues must have increased

Sauerstoffmangel erhoht bei Saugern je nach Dauer und Starke die Hamoglobinkonzentration bzw Sauerstoffkapazitat des Blutes Wahrend bei anhaltendem Sauerstoffmangel dieser Konzentrationsanstieg Folge einer erhohten Gesamthämoglobinmenge ist wird bei kurzdauernder Hypoxie und wahrend der ersten Tage in Hypoxie dieser Anstieg entweder auf einen Flussigkeitsverlust des Blutes im Sinne einer Hamokonzentration (SCHNEIDER 1921) oder auch auf einen Einstrom von Erythrozyten in die Zirkulation durch eine sogenannte Milzentspeicherung (BARCROFT 1927 SUNDSTROEM und MICHAELS 1942 KRAMER und LUFT 1949) zuruckgefuhrt

Vor allem von VERZÁR (1947) der die Auffassung vertreten hat dass Bilirubin oder andere Hamoglobinabbauprodukte adäquate Reize der Erythropoiese seien ist weiterhin die Frage einer hamolytischen Reaktion wahrend dieser hypoxischen Initialphase diskutiert worden

Tab I Plasmae weisskon_ent at on Hamoglob nkon_entration und H matokrit zo Normaltieren (I) Tieren nach 24—48 stund ger Hypoxie entsprechend 6 000 m Hohe (II) und Tieren wie II mit Corticosteroid Zusat (III) n = An_ hl der Tiere

	n	Pl sma w ss- k nz nt t on g	Hct	Hb g
I	10	5 44 ± 0 097	40 8 ± 0 56	13 1 ± 0 18
II	12	6 64 ± 0.275	47 1 ± 1 40	14 7 ± 0 42
III	10	6 69 ± 0 079	46 3 ± 0 6	14 4 ± 0 30

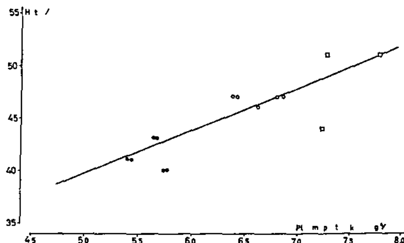
Die vorliegenden Untersuchungen behandeln vorwiegend die Blutver anderungen wahrend der ersten 48 Stunden nach einsetzender Hypoxie Es soll versucht werden aus dem Verhalten des Gesamthamoglobin der Hamoglobinkonzentration der Plasmaeiweisskonzentration und der spezifischen Aktivitat von mit C^{14} Glycin in vivo gezeichneten Erythrozyten eine Antwort auf folgende Fragen zu erhalten Ist die erhohte Hamoglobinkonzentration durch einen Flussigkeitsverlust des Plasmas zu erklaren liegt also eine Hamokonzentration vor? Wie verhalt sich das Gesamthamoglobin? Liegt eine hamolytische Reaktion vor?

Material und Methodik

D e Versuche wurden an mannlichen etwa 250—300 g schweren Wistarratten und Kragenratten ausgefuhrt Hypoxi wurde durch Unterdru k hervorgerufen dem d Tiere n kleiner n Unterdruckkammern wie f uhe bes hrieben (TRIBUKAIT 1962) aus gesetzt wurden Alle Untersuchungen selbst Blutentnahmen etc wurd n jedoch in M ereshohe unmittelbar n chd m d e T ere aus den Kammern genommen waren orge ommen D Futter bestand aus e nem speziellen Rattenb ot (Zusammensetzung s TRIBUKAIT 1960 a) Hafer Mohrruben und Wass ad l b t m

Die Gesamthamoglobinmeng u de m t e n mod fi ert n al eolaren CO Methode (TRIBUKAIT 1960 b) unter Berucks ht gung d pez ellen V erh ltnis e unt Hypoxie (TRIBUKAIT 1962) gemessen Hamoglob nkonzentrat o i und Hamatokrit aus denen si h zusamm n m t dem Gesamthamoglob n das Blut und Plasmavolumen be echnen, wurden n der fruher angegebenen Weise best mmt (TRIBUKAIT 1960 b) D e Bestim mung der Plasma weisskonzentrat on erfolgt na h d M kro-Kj ldahlmethode De von 30 Doppelproben berechn t V a ationsko ffiz nt b trug ± 4 3

Aufschluss uber e n ev ntu ll Hamolyse halt m n aus dem vorze tigen Abfall der R d o kt v t t n vi o gez chn t n H m ns W n f uh en Versu hen bei denen die Erythro tenlebens t best mmt wurde wurden da u mannl che Kragen atten (Nat on l Inst tut for Medical Resea ch M ll H ll London) verw ndet 10 T eren mit e nem Du chschnittsgewicht on 303 g ± 9 erh elten j etwa 10 μC (0 169 mg) C^{14} 2 Glycin i p R und 0 2—0 3 der rabfolgten Akt t t sind unter Normalbe di gungen b zum 40 T g na h d r Inj kt on wo der physiologische Erythro yten abbau und damit rbund n n Akt itatsabfall e nsetzt in rel tiv gle chbleibender Me ge im H min na hweisbar (FORSSBERG und TRIBUKAIT 1962 b) H min wurde n ch der von FORSSBERG und TRIBUKAIT (1962 a) beschri benen M kromethode isol ert und de en spezifische Akt tat g messen



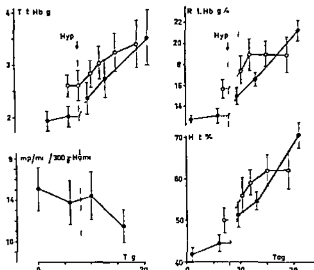
Verh lten von Plasmaeiw skonzentration und Hämatokrit von Ratten nach 24—48 stünd ger Hypox ntsprechend 6 000 m Hoh e ● = Normalt ere □ = Hypoxiet ere ○ = Hypoxie- t re m t Zusatz on Cortic teroid n Gleichung d r Regressionslin e $y = 4.04\,x + 19.51$ $r = 0.79$

Ergebnisse

In Tab I sind Mittelwerte der Plasmaeiweisskonzentration der Hamoglobinkonzentration und des Hamatokrit von Tieren nach 24—48 stundigem Aufenthalt in Hypoxie entsprechend 6 000 m Hohe zusammengefasst Diese sind gegenuber den Kontrollwerten hochsignifikant angestiegen ($p < 0.001$) Es finden sich also hier Veranderungen die allgemein als Zeichen einer Hämokonzentration gewertet werden

Wie weiter Abb 1 zeigt sind die Veranderungen der Eiweisskonzentration eng zu denen des Hamatokrit bzw der Hamoglobinkonzentration korreliert (Korrelationskoeffizient $r = 0.79$ $p < 0.001$) Aus Abb 1 geht ausserdem hervor dass die hypoxische Reaktion individuell sehr verschieden ausfallen kann einige Tiere reagieren nicht mit einem Anstieg der Plasmaeiweiss konzentration und auch nicht mit dem des Hamatokrit andere in ausgesprochenem Masse Corticosteroide (Prednisolon Natrium Succinat 1 25 mg/Tag i p) von denen ein der Steigerung der Plasmaproteinkonzentration entgegenwirkender Effekt fur moglich gehalten wurde hatten zwar keine derartige Wirkung die Mittelwerte von behandelten und nichtb handelten Tieren unterscheiden sich nicht signifikant von einander ($p > 0.05$) Es ist aber bemerkenswert dass die Reaktionsweise aller so behandelten Tiere auf Hypoxie wesentlich gleichmassiger wird

Abb 2 zeigt neben den Veranderungen der relativen Blutwerte und des Gesamthamoglobin von 2 Tiergruppen das Verhalten der spezifischen H min aktivitat wahrend der ersten Tage in Hypoxie entsprechend 6 000 m Höhe Die relativen Blutwerte bestätigen den bereits oben besprochenen Konzentrations



Verhalt n n Gesamt hämogl b spe ifi cher H m nakt vit t H mogl bink nzentration und Ha m tokrit w hrend in tialer Hypo t p chend 6000 m H h De Tag 0 in der rsten Versuchsserie (geschlossen Symbol) ent spricht dem 23 T g n h I j kt n von C 2 Glyc n Mitt lwert n j 10 T en m t $\pm \lambda\sigma$

anstieg während der ersten 48 Stunden der dann bei der einen Versuchsgruppe bereits deutlich in das Stadium einer sekundären Polyzythämie übergeht bei der anderen nach einigen Tagen zunächst zu einem Stillstand kommt

Die spezifische Häminaktivität ist nach 2 Tagen völlig unverändert und erst nach 8 Tagen hochsignifikant abgefallen ($p < 0\,001$) Nach 2 Tagen liegt also demnach weder eine messbare Hämolyse vor noch gelangt neugebildetes Hämoglobin in die Zirkulation in beiden Fällen sollte sonst nämlich die spezifische Aktivität abfallen

Demgegenüber wird wie auch aus Tab II für eine grössere Anzahl von Versuchen hervorgeht beim Übergang von 0 m auf 6 000 m Höhe eine signifikante Differenz der Hämoglobinmenge nach 48-stündiger Hypoxie gemessen Aber auch in 3 000 und 4 000 m Versuchen findet sich diese Differenz die trotz des relativ geringen Umfangs der einzelnen Gruppen eine klare Signifikanz aufweist Lediglich in 5 000 m ist der Unterschied nicht zu sichern Es ist weiterhin bemerkenswert dass die Höhe dieser Differenz relativ unabhängig vom Grad des Unterdrucks ist

Aus Tab II sind weiterhin Daten von Tiergruppen ersichtlich bei denen die Erythropoiese bereits zuvor erhöht war entweder durch Unterdruck (Versuch Nr 1957 IV V 1958 III) oder durch Anämie (Versuch Nr 1958 IV V) Die Tiere in 4 000 m waren zuvor etwa 2 Monate in 2 000 m die Tiere in 5 000 m etwa 2 Monate in 4 000 m und die Tiere in 6 000 m etwa 2 Monate in 3 000 m Die Anämie war durch Entnahme etwa 1/3 des Blutvolumens 18 Tage zuvor hervorgerufen worden Beim Übergang von 2 000 m auf 4 000 m und von 4 000 m auf 5 000 m also einem relativ geringen Unterschied zwischen Ausgangslage der Erythropoiese und zusätzlichem Reiz findet sich keine

Tab II Gesamthamoglobin von Tieren vor Hypoxie (Tag 0) und nach 2 Ta en in verschieden starker Hypoxie mit Differenz und Signifikanz der Differenz Die Tiere der ersten Versuchsserien (1957 I—1961 I) waren direkt von 0 m den entsprechenden Unterdruckstufen ausgeset t worden Bei den zweiten Versuchsserien (1957 IV—1957 V) befanden sich die Tiere zu or in ?000 m 4 000 m b w 3 000 m Hohe bei den letzten Versuchsserien (1958 IV—1958 V) war d e Erythropoiese der Tiere durch eine akute Blutung 18 Tage vor Hypoxiebe n stimul ert word n n = Anzahl der Tiere

Versuch Nr	n	H he m	Total Hb g		D ff	Diff	t D ff	p
			Tag 0	T g 2				
1957 I	5	3 000	3 09 ± 0 115	3.25 ± 0 119	0 15 ± 0 064	5 0	2 375	0 05–0 01
1958 I	6	4 000	2 3 ± 0 113	2 68 ± 0 140	0 34 ± 0 079	14 4	4 ?62	0 01–0 001
1957 II	5	4 000	2 72 ± 0 104	2 98 ± 0 083	0 27 ± 0 051	10 1	5 296	0 01–0 001
19 7 III	5	5 000	3 34 ± 0 140	3 63 ± 0 208	0 29 ± 0 191	9 0	1 516	> 0 05
1959 I	9	6 000	2 47 ± 0 033	2 78 ± 0 075	0 32 ± 0 061	12 7	5 214	< 0 001
1958 II	6	6 000	2 04 ± 0 090	2 40 ± 0 069	0 35 ± 0 044	17 8	7 986	< 0 001
1959 II	9	6 000	2 67 ± 0 125	3 01 ± 0 100	0 34 ± 0 0?0	13 6	5 755	< 0 001
1959 III	10	6 000	2 60 ± 0 091	2 83 ± 0 082	0 22 ± 0 057	8 9	3 850	< 0 001
1961 I	10	6 000	2 00 ± 0 061	2 35 ± 0 086	0 35 ± 0 051	17 7	6 863	< 0 001
1957 IV	6	4 000	3 16 ± 0 180	3 14 ± 0 185	–0 015 ± 0 1099	0 27	0 136	> 0 05
1958 III	6	5 000	3.21 ± 0 164	3 34 ± 0 222	0 122 ± 0 0974	3 52	1 25?	> 0 05
1957 V	5	6 000	3 66 ± 0 08?	3 93 ± 0 136	0 276 ± 0 0758	7 46	3 ?41	0 05–0 01
1958 IV	7	6 000	1 97 ± 0 074	2 21 ± 0 083	0 24? ± 0 08?4	12 7	2 937	0 05–0 01
1958 V	7	6 000	2 06 ± 0 059	2 27 ± 0 075	0 210 ± 0 0775	10 6	2 710	0 05–0 01

statistisch zu sichernde Differenz Beim Übergang von 3 000 m auf 6 000 m ist der zusatzliche Reiz bedeutend grosser und es lasst sich auch eine deutliche Differenz nachweisen Bei den Anamiegruppen ist der hypoxische Effekt zwar statistisch signifikant jedoch verglichen mit den Normaltieren deutlich abgeschwacht

Diskussion

Die Untersuchungen bestatigen die verschiedentlich beschriebene initiale Veranderung der Plasmaeiweisskonzentration unter Hypoxie (SUNDSTROEM und MICHAELS 1942 HURTADO MERINO und DELGADO 1945 ASMUSSEN und NIELSEN 1945 DUNER PERNOW und TRIBUKAIT 1958) In enger Korrelation zur Plasmaeiweisskonzentration steigt dabei der Hamatokrit und es ist durchaus naheliegend einen ursachlichen Zusammenhang zwischen den Änderungen des Hamatokrit und der Plasmaeiweisskonzentration anzunehmen Rein quantitativ stimmen die gefundenen Veranderungen mit den berechneten einigermassen uberein Steigt wie im gegebenen Fall die Plasmaeiweisskonzentration von 5 44 g % auf 6 64 g % sollte das Plasmavolumen um 18 % sinken und der Hamatokrit um 22 % steigen Der gefundene Hamatokritanstieg betragt 15 %

Welche Prozesse im Einzelnen den Anstieg der Plasmaeiweisskonzentration veranlassen ist unklar Es erscheint aber naturlich dass auch das Plasma bei dem 15 %igen Wasserverlust den der Organismus wahrend der hypoxischen Initialphase erleidet (PICON REATEGUI *et al* 1953) mit beteiligt ist Diesem Wasserverlust scheint eine Flussigkeitsverschiebung vom Plasma zum interstitiellen Flussigkeitsraum voranzugehen (ARNOULD und LAMARCHE 1952) Beim Meerschweinchen hat weiter Histamin offenbar eine gewisse Bedeutung unter Hypoxie ist ein kraftiger Histaminanstieg im Blut nachweisbar der zur Plasmaproteinkonzentration bzw zum Hamatokrit hochsignifikant korreliert ist Antihistamin normalisiert wenigstens teilweise die Plasmaproteinkonzentration (DUNER PERNOW und TRIBUKAIT 1958) Bei der Ratte dagegen andert sich das Bluthistamin offenbar nicht (Kontrollen 0 054—0 040 μg/ml Blut n = 3 Hypoxietiere 0 057—0 044 μg/ml n = 3) Antihistamin (Pyribenzamin 1 mg/Tag i p) ist ohne signifikanten Effekt auf Plasmaeiweisskonzentration oder Hamatokrit

Eine initiale Hamolyse liegt soweit sie sich aus einem Abfall der spezifischen Haminaktivitat erkennen lasst nicht vor VERZARS Hypothese von Bilirubin oder anderen Abbauprodukten des Hamoglobins als adaquater Reiz der Erythropoiese findet also nach den vorliegenden Ergebnissen dieser allerdings ziemlich groben Methode keine Stutze jedenfalls nicht unter diesen Versuchsbedingungen Untersuchungen des Bilirubin unter Hypoxie haben auch im Hinblick auf eine eventuelle veranderte Leberfunktion ebenfalls nicht eine Hamolyse einwandfrei nachweisen konnen (VERZAR *et al* 1933 MERINO 1950 weitere Literatur s HIRSJARVI 1953) Die Moglichkeit dass radioaktives Hamin nachgebildet wird und damit die spezifische Aktivitat steigt kann definitiv verneint werden Bei der Konkurrenz der verschiedenen Syntheseprozesse um Glycin erhalt Hamin nur den sehr bescheidenen Anteil von 0 2—0 3 % Eventuelle freiverfugbare Spuren radioaktiven Glycins 30 Tage nach dessen Injektion gehen entsprechend auch nur in einer Menge von 0 2—0 3 % in die Haminsynthese ein und sind ohne jede praktische Bedeutung

Die unveranderte Haminaktivitat 48 Stunden nach einsetzender Hypoxie bedeutet weiter dass der Zirkulation wahrend dieser Zeit kein neugebildetes Hamin zugeführt worden ist Das entspricht auch dem etwa 48-stundigen Intervall zwischen Beginn des Sauerstoffmangels und einsetzender Retikulozytose (SUNDSTROEM und MICHAELS 1942 SAATHOFF 1950 a MERTENS 1957 MÖLLER 1960) Aus derartigen Daten die Schlussfolgerung zu ziehen (ERSLEV 1959) dass die Proliferation der Erythropoiese an den Stammzellen einsetzt und dass 48 Stunden notwendig sind bis eine neugebildete Zelle alle Teilungs und Reifestadien durchlaufen hat und in die Zirkulation gelangt kann jedoch trugerisch sein Teils scheint ein verkurzter Entwicklungsweg mit einem unmittelbaren Übergang vom Erythroblasten zum Erythrozyten bzw Retikulozyten moglich zu sein (WITTSCHINSKI 1927 ALPEN und CRANMORE 1959) teils scheint an allen teilungsfahigen Zellen besonders an den reifsten Zell

elementen die Proliferation zu einem sehr frühen Zeitpunkt der Hypoxie einzusetzen (MÖLLER 1960), wodurch sich das erythropoietische Gewebe während der ersten 48 Stunden mit Retikulozyten in starkem Masse auflad (SAATHOFF 1950 b).

Die vorliegenden Werte der Gesamthämoglobinmenge, die aus einem zirkulierenden und aus einem geweblich fixierten Anteil besteht, vermittelt eine gute Vorstellung von der hohen quantitativen Bildungsleistung, die das erythropoietische Gewebe gerade während dieser initialen Hypoxieperiode vollbringt. Die Stimulation der Erythropoiese, gemessen an der gebildeten Hämoglobinmenge, scheint zunächst mehr oder minder unabhängig von der Stärke des hypoxischen Reizes zu sein. Erst im weiteren Verlauf ergibt sich dann ein je nach Hypoxiegrad differenziertes und charakteristisches Bild (TRIBUKAIT 1962). Es ist weiterhin natürlich, dass bei bereits stimulierter Erythropoiese mit einem relativ aktiven erythropoietischen Gewebe wie beim Übergang von einer niedrigeren zu einer höheren Hypoxiestufe oder auch nach einer Blutungsanämie der Zusatzreiz nur einen geringen Effekt hat.

Die Frage, welche Prozesse den Abstrom der reifen Blutelemente vom erythropoietischen Gewebe in die Zirkulation steuern bzw. wodurch es zu dieser Zellansammlung im erythropoietischen Gewebe kommt, muss offen bleiben. LAMERTON, BELCHER und HARRISS (1959) versuchen eine beträchtliche Ansammlung von relativ reifen Zellen im Knochenmark von Ratten 1—2 Tage nach einer Röntgenbestrahlung auf den fehlenden Populationsdruck („growth pressure") der normalerweise vorhandenen jüngeren Zellelemente zurückzuführen. Diese Vorstellung wird jedoch von STOHLMAN (1960) abgelehnt. Die Nahrungskarenz der Tiere während der hypoxischen Initialphase mag auch eine Bedeutung haben. Beim normalen Tier wird dadurch der Retikulozytenübertritt in die Zirkulation gehemmt, während eine darauffolgende Nahrungszufuhr eine Retikulozytenausschwemmung verursacht (MEYER, THEWLIS und RUSCH 1940). Schliesslich ist auch an rein zirkulatorische Faktoren zu denken.

Die eingangs gestellten Fragen lassen sich nach den vorliegenden Untersuchungsresultaten folgendermassen beantworten: Der während der ersten 48 Stunden unter Hypoxie festzustellende Anstieg der relativen Blutwerte wird durch einen Flüssigkeitsverlust im Sinne einer Hämokonzentration hervorgerufen. Gleichzeitig steigt die Gesamthämoglobinmenge, das gebildete Hämoglobin bleibt jedoch im erythropoietischen Gewebe liegen und tritt noch nicht in die Zirkulation über. Eine hämolytische Reaktion scheint nicht vorzuliegen. Die Milz mit einem Gewicht von etwa 0,5 g scheidet als Blutspeicher in diesem Zusammenhang schon aus quantitativen Gründen von vorn herein aus.

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From the Department of Physiology Univers ty of Goteborg S eden

Cardiovascular Responses in "Diving" and During Brain Stimulation in Ducks

By

ERIC FEIGL and BJORN FOLKOW

Rec ved 21 June 196

Abstract

FEIGL, E and B FOLKOW *Card ovascula response n d n g and duri bra st mul t n in ducks* Acta physiol scand 1963 57 99—110 — The circulatory response in the d ving reflex as in d cated by ch nges in heart rate of unanestheti ed ducks was found to depend on more than the asphyxia of submersion D ving bradycardia was found to result from at least three factors these we e

I A specific nervous eflex resulting from submers on of the head. This first factor is the permiss e an l necessary factor for establ h ng the div ng eflex but t s strongly reinforced by at least two other me hanisms

II The p ogressi e hypercapnia during the asphyxia

III The ncreas ng anoxia during he asphyx a

The interrelat onsh ps of thes three f tors are briefly discussed Sti mulat n of an a n the mes ncephalon n many respects clos ly es mbles tl specific central permiss e fa tor needed f r the d ing flex

A second a n th d encephalon was found f om which chol n e gi asod l tor fibers to the muscle vascular bed could be activated

Profound cardiovascular changes form one of the prominent aspects of the response to submersion exhibited by many aquatic animals The reflex brady cardia is an especially striking phenomenon Both diving mammals and diving birds show a pronounced slowing of the pulse when submerged Diving in mammals has been reviewed by IRVING (1939) The cardiovascular changes upon submersion of birds especially the bradycardia have been studied by SCHOLANDER (1940) and ELIASSEN (1960)

This study was carried out to investigate the relationship of some of the factors which are involved in the onset intensification and elimination of the cardiovascular response during submersion in birds In Part I the diving response in unanesthetized ducks was studied In Part II an attempt was made to mimic the responses seen in the unanesthetized animals by producing topical mesencephalic stimulations in anesthetized ducks

Part I Development of bradycardia in the 'diving reflex' of ducks

Method Thirteen adult white common domestic ducks (*Anas boscas*) were used A tracheal cannula was inserted using local anesthesia and arranged so that respiratory valves and a respiratory pump could be attached to it Special care was taken to keep the respiratory dead space as small as possible always less than 3 ml A fixed stroke volume respiratory pump of the Starling type was used and adjusted in rate and tidal volume to approximate the normal quiet breathing of the bird Whenever comparisons were made using the respiratory pump the stroke volume and rate were kept constant throughout the experiment Various gas mixtures were made up using a Contimeter gas flow meter and were kept in plastic Douglas bags The bags could be connected to the inlet of the respiratory pump or to a one way respiratory valve and thus to the duck's trachea A standard electrocardiograph (Elema Schonander Mingograph) was used to record the heart rate In most animals the respiratory movements were also recorded by a pneumograph connected to one of the channels of the Mingograph

The duck was placed on the experimental table facing a basin of water so that its head could be dipped under water leaving the tracheal cannula above water This arrangement permitted various respiratory maneuvers to be performed with the duck in a diving (head submerged under water) or head up position The diving period usually lasted 1 to 1 5 min and was standardized as far as was possible

Because ducks vary quantitatively — though not qualitatively — in their responses to submersion the experiments were designed so that comparisons were made only within individual ducks not between ducks In each duck two or more experimental procedures were compared The experimental trials were repeated in an alternating fashion so that any long term effects of the procedure cancelled out The results of the various trials in a single duck were averaged and compared using Student's t test

Results The onset of bradycardia after submerging the duck's head had a characteristic pattern There was an immediate reduction in pulse rate at the moment the head was placed under water This immediate response was usually of the order of a 20 % reduction in the heart rate After the initial fall the pulse rate continued to decrease until very low values were reached If the duck moved or struggled during the dive the heart rate often decreased in a small step after each brief bout of moving Representative time courses of developing bradycardia are illustrated by the unbroken lines in Fig 1 and 2 Different ducks varied greatly in the extent to which they developed bradycardia upon submersion There was also considerable variation in the initial bradycardia response from duck to duck The bradycardia of submersion was promptly broken when the head was raised from the water and the duck

300
250
200
150
100
50
HEART RATE BEATS PER MIN
AIRWAY CLOSED
SUBMERSION
5 30 45 60 75 SEC

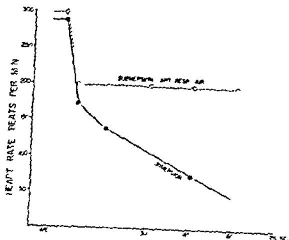
Fg 1 2 6 kg du k ch p nt ep es nts erag of 10 d t r mm t on Th ff ct f a 1 m n ut period f asphyxia by oc lud g the t h l annul and by bm rs n of th h ad a c m p ed Bra k ts nd c t ± S E

breathed again The increased heart rate developed in the first one or two beats immediately after the dive was over and was often more rapid than the control value

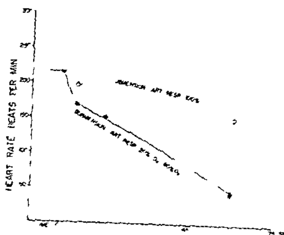
The bradycardia of submersion was not due to simple asphyxia This was demonstrated in the following way Ducks were subjected to a 1 minute period of asphyxia either by dipping the head under water or by occluding the tracheal cannula with the head up Simple respiratory obstruction with the head up gave some fall in heart rate but the extent was far smaller for a given period of asphyxia than when the head was submerged Further respiratory obstruction did not cause the sharp initial decrease in heart rate een when the head was dipped under water Fig 1 compares a 1 minute period of asphyxia caused by tracheal obstruction with the head up and a similar period of submerging the head under water for a representative duck The average heart rate in this duck after one minute of tracheal occlusion fell from 260 to 156 The average rate after a 1 minute submersion fell from 273 to 18 beats per minute This difference is highly significant ($p < 0.001$)

The important role of submersion as distinct from asphyxia was also shown by submerging the duck s head for a minute or so and then raising its head but preventing it from breathing by having the tracheal cannula occluded When this was done the heart rate increased as soon as the head came out of the water It rose to about 75 % of the control value within one or two beats When the airway was opened the pulse rose to control values or higher This again indicates that submersion itself not just asphyxia was important in the development of bradycardia in diving unanesthetized ducks

If asphyxia with the head above water gives very little bradycardia does asphyxia play any role in diving bradycardia? This question was examined in the experiment shown in Fig 2 In this experiment the duck s head was submerged as usual but on alternate submersions the animal was artificially



Fg 2 2 9 kg duck each po t epresents an averag of 7 deter minat ons On minut submers ons with and witho t art ficial resp rat on using a are compared. Brack ts nd cate ± S E



Fg 3 ɔ kg duck each pr t represents an a erage of 10 deter minat ons One minute submer sions w th art ficial resp at n using d fferent gas m xtu s Art f cial resp ration w th 100 N demon strates th eff ct of noxia w th out hypercapnia Art f c al resp rat on w th 20 CO 80 O dem nstrates th ffect of hyper cap a w th ut an ia. Bra kets indicate ± S E

ventilated with air durinɡ the dive In seven submersions with artificial respiration with air for one minute the pulse rate decreased from 300 to 201 Seven submersions with asphyxia for one minute gave an average final value of 45 This is a highly significant difference ($p < 0{\cdot}001$) The moderate initial bradycardia was accentuated by asphyxia This demonstrated that not only was submersion important to the development of bradycardia but also that the asphyxia of submersion was an important factor potentiating the immediate bradycardiac response

It then remained to be investigated which aspects of the asphyxia were most important the lack of oxygen or the accumulation of carbon dioxide An experiment comparing the effects of anoxia versus carbon dioxide is illustrated in Fig 3 The duck s head was dipped under water and during the submersion the animal was artificially ventilated On alternate runs the gas mixture used was pure nitrogen and the other a mixture of 20 % CO_2 80 % O_2 The com

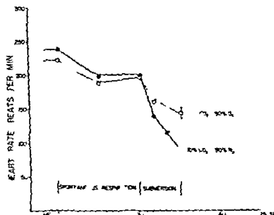


Fig 4 4 kg duck each point represents an average of 10 determinations The duck was prev ntilated for 30 sec with different gas mixtures and then submerged P entil t on with 10 CO 90 O t give hypercapnia s omp ed with 10 CO 90 N to give both hypercapnia and noxia Brackets indicate ± S E

bined effect of submersion and anoxia but without the accumulation of CO_2 was demonstrated when the duck was ventilated with pure N An initial decrease was seen upon submersion but the subsequent fall in heart rate was small decreasing from 220 to 155 after 1 minute When the animal was artificially respired with 20 CO_2 in O_2 during submersion heart rate decreased from 217 to 48 after 1 minute This is a highly significant difference ($p < 0.001$) To illustrate that carbon dioxide accumulation seems to be more important in potentiating the initial diving bradycardia than anoxia *per se* the following experiment was performed The duck was forced to "dive for a minute and at the moment of the dive the tracheal cannula was occluded During the last 10 sec of the submersion the tracheal cannula was opened and the lungs were artificially ventilated with either 100 % nitrogen or 10—20 carbon dioxide in oxygen Ventilation with nitrogen which would enhance the prevailing hypoxia but wash out the accumulated CO_2 almost immediately increased heart rate at the first filling of the lungs Artificial ventilation with carbon dioxide in oxygen which would maintain or aggravate the hypercapnia but temporarily eliminate the hypoxia generally did not interrupt the intense bradycardia after a minute of submersion.

Anoxia also is of importance This was demonstrated in a slightly different sort of experiment The duck was made to breath various gas mixtures from Douglas bags spontaneously for a 30 sec period before submersion Fig 4 demonstrated the results of an experiment in which the effects of a 10 % CO_2 in O_2 mixture were compared with a 10 % CO in N_2 mixture in such a prebreathing experiment After prebreathing with 10 % CO_2 in oxygen and submersion for 15 sec the heart rate fell to 147 Prebreathing with 10 % CO in N to give anoxia followed by 15 sec of submersion gave a heart rate of 93 This difference is highly significant ($p < 0.001$) The experiment demonstrates that anoxia with CO_2 is a definitely stronger stimulus for bradycardia

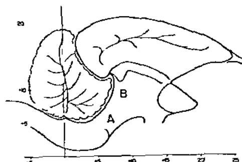
than just CO_2 Fig 4 also demonstrates that spontaneous respiration with gas mixtures containing CO_2 or even CO_2 in N_2 for 30 sec slows the heart very little in ducks Only a slight bradycardia was found even when the ducks were made to breathe such mixtures for a minute or more as long as their heads were up Addition of a dive produced an immediate intense brady cardia

It should be pointed out that the results have been presented as a series of representative experiments in individual ducks The differences were not always as great as those illustrated On the other hand the differences were always found to be in the same direction The experiments were repeated in a number of ducks often with different time intervals and slightly different gas concentrations but in principle the results were the same

To summarize submersion of the head produces an immediate moderate bradycardia which is much accentuated by the gradually developing asphyxia Both the anoxia and the hypercapnia are of importance in potentiating the initial diving reflex response Anoxia and hypercapnia without submersion have little influence on the heart rate Hypercapnia appears to be of more dominating importance than anoxia in potentiating the bradycardia

Discussion The present findings are in agreement with the previous literature describing submersion bradycardia in ducks A fall in heart rate of 50 % or more was usually seen to develop in the first minute of submersion The sharp initial decrease in the heart rate of about 20 % within the first few seconds of submersion is evidence that submersion *per se* is an important factor in producing reflex slowing of the heart since the degree of asphyxia within a few seconds must be quite small The importance of submersion in the ability of ducks to withstand asphyxia has long been recognized Also that ducks develop a more pronounced bradycardia with submersion than with tracheal occlusion has been previously reported by HUXLEY (1913 c) who reviews the earlier literature

These results appear to indicate that the development of bradycardia in diving ducks is dependent on at least three factors The first factor is associated with submersion of the head This may be considered as a specific central nervous reflex which results from submersion Probably a number of modalities contribute to this central nervous reflex which is associated with submersion of the head HUXLEY (1913 a b) and PATON (1913) have presented evidence that the labyrinths and proprioceptors in cervical muscles are important in the duck s ability to dive Certainly the present procedure of dipping the head would stimulate receptors in labyrinths and cervical muscles It also seems likely that receptors in or about the respiratory tract are involved as the diving response can be elicited by tipping the animal slightly on a board and submerging only the nares In any case it would be expected that there is a wealth of sensory information available to the central nervous system which could result in a specific reflex This reflex is all important to the development



Fg 5 P lim n ry m p f the du k bra n h wing a A in th mesen ph al n and a B n th d en ephalon

of diving bradycardia since a marked slowing of the heart will not occur without it even if a severe asphyxia is produced

The second and third factors are concerned with the asphyxia of submersion The results indicate that if the first factor the specific central nervous reflex is present both hypercapnia and anoxia contribute to the circulatory adjust ment as measured by the extent of bradycardia Hypercapnia and anoxia may thus be considered potentiating second and third factors Hypercapnia appears to be the stronger of these two factors but the combination of hyper capnia and anoxia gave the strongest effect by far

Part II Cardiovascular effects of brain stimulation in ducks

M thod Fifteen adult hite domestic du ks (*Anas b as*) ere used The du ks were anesth ti ed with p ntobarbital 30 mg/kg body v ght ntraven usly A tracheal cannula as inserted and variou resp atory proc du es ere carried out as n Pa t I

The kull was op ned th a dental drill and th h ad pla d n a Horsley Clarke st reotaxic app atu The u ual t reotaxic instrument used for ats was modifi d sl ghtly so th t th du k s h d ith ts late ally pla ed y could be a ommodat d Th e o h ns ntal plan as hosen as th lin b twe n the ea ba and the nfer or m gin of th nf ao bit l ridge t th most ant rio orner of the ey The electrodes were pos t oned in th bra n for a ute timul tio with th a d of a p el minary ste eo taxi m p of the du k b a n Th map was p ep ed f om section fi ed with f rm l n n s l n The timulat d po nts we e dentified nd ma ked n the map (ee F g 5) No h tology as done

Electrolyt cally harpen d st el monopola el ctrodes nsulat d with sp c al elect ical in ulat ng v rn h wer used The usu l procedur was to use pa ed el ctrodes st mu lating the s me rea of the bra n s multaneously on both s des Rectangula cathodal timulat on wer del vered from a Grass mod l S 4- A timulator The usu l stimulation cha a teri tics e e a frequen y of 70 per sec a pulse duration of 2 mse and between 2 nd 3 olts del ered to th ele trodes n p rallel

H pa n 2 5 mg per kg body we ght v as gi en and blood pressu was meas ed usi g me cury manomet r from the wing art ry of the duck. Blood flow f om the h d leg of the d k 'as measured in th f moral e n and turned via th jugular vein The c rculation below th ankle was in most experiments completely excluded

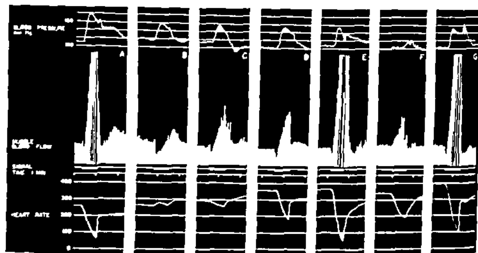


Fig. 6. 3.1 kg duck. Pentobarbital anesthesia. a) Stimulation in area A in the mesencephalon. b) Stimulation in the same point with artificial respiration using air. c) Stimulation in the same point with artificial respiration using 100% N_2. d) Stimulation in the same point with artificial respiration using 20% CO_2 80% O_2. e) Stimulation in the same point with artificial respiration using 20% CO_2 80% N_2. f) Tracheal airway closed, no stimulation. g) Final stimulation without artificial respiration.

by a very tight rubber tourniquet so that blood flow in the femoral vein was preponderated from the limb muscles. This preparation is here described as a "muscle flow" though some small fraction must come from the skin of the thigh. Blood flow was measured in a transparent drop chamber filled with silicone oil (LINDGREN 1958). The drops were counted with a photocell drop counter which activated an ordinate writer so that the height of the ordinate was proportional to the period between drops (CLEMENTZ and RYBERG 1949). That is, the height of the ordinate inscribed is inversely proportional to flow. Heart rate was measured using an electromanometer (Elema) connected to the other wing artery. Arterial pulsations were then recorded at a fast paper speed using a Mingograph recorder. Respiratory movements were monitored using a pneumograph recording on the Mingograph.

Results. On exploration of the base of the brain using stimulating electrodes two areas were found which gave striking cardiovascular effects. These are shown in Fig. 5 as areas A and B. Area A is in the mesencephalon and area B is in the diencephalon, both areas are near the midline. The stimulations were most effective when the electrodes were 1 to 2 mm lateral to the mid sagittal plane. Areas caudal to the mesencephalon were not explored.

Stimulation in area A resulted in a rise in blood pressure, a marked fall in heart rate and an intense vasoconstriction in the muscle bed. Upon stimulation the bird stopped respiring and remained apneic until the stimulation was stopped. Representative responses are illustrated in Fig. 6 parts A and G.

If apnea during these stimulations was prevented by artificial respiration with air, the cardiovascular responses were greatly changed. The brady-

cardia did not develop and the muscle vasoconstriction was absent or greatly decreased The blood pressure increase was not great This is illustrated in Fig 6 part B When apnea during a stimulation was prevented by artificial respiration with 100 % N_2 or mixtures of O_2 and CO containing as much as 20 % CO in O_2 the cardiovascular effects were greatly reduced (see parts c and d in Fig 6) When the birds were artificially ventilated with a mixture of 20 % CO_2 80 % N during a stimulation marked cardiovascular changes were seen A pronounced bradycardia and muscle vasoconstriction developed and a rise in blood pressure was observed This is illustrated in part E of Fig 6 The extent of these changes was of the same magnitude as when there was no artificial respiration so that the apnea caused by the stimulation was maintained Compare parts A and E of Fig 6 It should be stressed that these circulatory changes are not only due to the inhalation of a mixture containing CO and N_2 because spontaneous respiration of 20 % CO 80 % N_2 for one minute did not result in much change in the cardiovascular variables recorded Simple asphyxia does not produce the marked changes observed during stimulation as illustrated in part F of Fig 6 This again points to the importance of the mesencephalic stimulation

The circulatory response to stimulation in area A could be nearly stopped during stimulation if the bird was given a few breaths of artificial respiration This artificial respiration was usually not effective in breaking the response if the gas mixture contained 10 per cent or more of CO Artificial respiration with nitrogen on the other hand would greatly reduce the response as was observed in the awake ducks during diving

Stimulation in area B is of some interest as it resulted in a marked increase in muscle blood flow Characteristically the heart rate increased somewhat and the blood pressure rose Respiration during the stimulation varied sometimes slowing sometimes increasing or becoming irregular Atropine prevented the vasodilatation Fig 7 illustrates an experiment in which this area was stimulated before and after atropine The blood pressure rise was enhanced and the heart acceleration still remained after atropine

In summary stimulations in area A in the mesencephalon resulted in complete apnea accompanied by a rise in blood pressure pronounced bradycardia and a striking vasoconstriction in the muscle bed This response could be nearly eliminated if the apnea were prevented by artificial respiration with air Ventilation during the stimulation with a gas mixture containing CO and N did not prevent the response Asphyxia alone did not mimic the centrally induced response After a circulatory response was well established by stimulation it could be greatly reduced by one or two breaths if the gas used did not contain CO

Stimulation in area B in the diencephalon resulted in an intense vasodilator response in the muscle vascular bed which was blocked by atropine leaving other parts of the cardiovascular response little changed

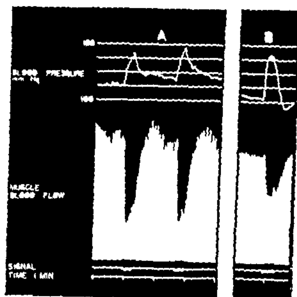


Fig 7 2 8 kg duck pentoba b tal anesth es a) St mulat on in are B in the d enc phalo sh w ng muscle a odil tatio b) Stimula t n in the same po nt after 0 5 mg/kg bodyweight has be n g e

Discussion Stimulation of area A in the brain resulted in complete apnea marked bradycardia strong muscle vasoconstriction and a rise in blood pressure This cardiovascular adjustment generally resembles that seen in the diving reflex of ducks with some exceptions The initial sharp decrease in heart rate seen when the head is submerged is not seen when the mesencephalic stimulation is turned on This does not appear as a large discrepancy since it is unlikely that a gross stimulation in the brain should be able to exactly mimic a delicate physiological state in the brain On the other hand the bradycardia elicited by stimulating in area A resembles the bradycardia of submersion in many other respects Diving bradycardia was seen to be dependent on three factors in the first part of this report They were 1) a specific central nervous reflex resulting from submersion 2) the accumulation of CO in the resulting asphyxia and 3) the anoxia of asphyxia The results in part II suggest that a stimulation in area A can in some respects substitute for factor I (the specific central nervous reflex) in the development of bradycardia in anesthetized ducks

Marked bradycardia could not be elicited from unanesthetized ducks by asphyxia unless the head was submerged Simple asphyxia or breathing mixtures of CO_2 and N_2 did not affect the cardiovascular system much in unanesthetized ducks as indicated by the heart rate The same was true for anesthetized ducks where heart rate blood pressure and muscle blood flow were measured Only when topical mesencephalic stimulation was present with asphyxia were sharp circulatory changes observed In unanesthetized ducks the central nervous reflex was evoked by submerging the head in

anesthetized ducks this was done by stimulating in area A. The correspondence between the various factors involved in the development of bradycardia in part I and II of this study are in good agreement. The relative effects of hypercapnia and anoxia seem to be very nearly the same during submersion or stimulation in area A.

Muscle vasoconstriction during diving has been reported in ducks and other diving birds by SCHOLANDER (1940) using serial lactic acid determinations to evaluate the changes in muscle blood flow. ELIASSEN (1960) using lactic acid determinations and a heat loss technique to evaluate flow did not confirm Scholander's finding of muscle vasoconstriction during submersion in diving birds. Scholander answered Eliassen's criticisms in a later paper (SCHOLANDER *et al.* 1962). JOHANSEN and KROG (1958) observed muscle vasoconstriction during submersion in ducks using a venous occlusion technique to estimate blood flow. Muscle vasoconstriction during diving has been reported in a number of other species and is reviewed by SCHOLANDER *et al.* (1962). Despite some evidence to the contrary it seems most likely that muscle vasoconstriction is part of the diving reflex in ducks. The muscle vasoconstriction elicited by stimulation in area A appears consistent with a diving response.

The increase in blood pressure observed during stimulation in area A is not in perfect agreement with what has been recorded in diving reflex of birds. JOHANSEN and KROG (1958) measured femoral artery pressure in ducks during submersion and found that arterial pressure was maintained. A sharp rise in pressure was not seen. ELIASSEN (1960) measured arterial pressures in a number of diving birds and also found that the blood pressure was maintained but did not observe significant increases in blood pressure, but some of his records do show a small increase in pressure during the dive. While the increase in blood pressure seen with stimulation in area A is probably greater than is expected with diving it is significant that despite a marked bradycardia the arterial pressure does not fall. Severe bradycardia without a fall in blood pressure is indicative of a special sort of cardiovascular adjustment which is found in diving and also is characteristic of stimulations in area A. The blood pressure responses during these stimulations are therefore in general agreement with what occurs in a dive. Further studies including ablation procedures will be needed to establish the role of this area in the diving reflex of ducks.

The results of stimulation in area B is of some interest as it in several respects markedly differs from that elicited from area A. On stimulation of area B a pronounced dilatation of the muscle vascular bed was observed which is most easily interpreted as activation of cholinergic dilator fibers. This effect is selectively blocked by atropine as are the cholinergic dilators in other species. This muscle vasodilatation is combined with a sympathetic excitation in other parts of the cardiovascular system as indicated by the cardiac acceleration and the blood pressure rise. The cholinergic vasodilator system has been extensively reviewed by UVNÄS (1960). ABRAHAMS, HILTON and ZBROZYNA (1960) have

studied the reaction pattern which accompanies central nervous system activation of the muscle vasodilator fibers and equate this with the defence reaction The present study provides no information as to whether active cholinergic vasodilatation is part of the defence reaction in ducks but the response pattern induced from area B is closely similar to the defence reaction of the cat In fact it is not at all clear what the defence reaction in ducks might be There are certainly situations of alarm where a diving bird will dive calling for the diving reflex and situations where it flies away where the cardiovascular pattern produced from area B would seem more suitable

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Dissociation of Contraction and Stimulation of Lactic Acid Production in Experiments on Smooth Muscle under Anaerobic Conditions

By

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Abstract

LUNDHOLM, L. and E. MOHME-LUNDHOLM. *Dissociation of contraction and stimulation of lactic acid production in experiments on smooth muscle under anaerobic conditions.* Acta physiol. scand. 1963. 57. 111—124. — In experiments on bovine mesenteric arteries under anaerobic conditions tone-promoting drugs gave rise to contraction and to augmentation of the lactic acid production. Under certain experimental conditions it was possible to dissociate these effects. In the presence of glucose dihydroergotamine selectively inhibited the contractile effect of adrenaline without influencing its stimulatory effect on lactic acid production. Replacement of the Tyrode solution with 6 per cent dextran solution resulted in selective inhibition of the contractile effects of adrenaline and histamine but did not interfere with the stimulation of the lactic acid production. Potassium ion selectively stimulated the contractile process while the lactic acid production diminished during the contraction phase *per se*. On substrate-depleted arterial muscle potassium ions had a more pronounced stimulatory effect than did adrenaline, histamine or barium ions. It is assumed that despite the correlation of the contractile effects of tone-promoting drugs to the stimulation of lactic acid production these two factors are activated by separate mechanisms.

In an earlier investigation the energy metabolism associated with isotonic contraction of vascular smooth muscle was calculated from the lactic acid production under anaerobic conditions (LUNDHOLM and MOHME-LUNDHOLM 1962 a). A conspicuous correlation emerged between the magnitude of contraction and the stimulation of lactic acid production resulting from addition of various contracting drugs as well as from electrical stimulation. With substrate depletion

Table I Effect of adrenergic blocking drugs on the stimulating action of adrenaline (1 10⁻⁴ e try

Drug conc	Glucose per cent	Duration min	n	Difference in lactic acid production (mg/100 g)	
				Adr Control	Drug Control
DHE (1 10⁻⁶)	0	45	9	13 5 ± 4 2 P < 0 05	11 1 ± 4 6 P < 0 05
DHE (1 10⁻⁶)	0.5	120	7	27 9 ± 5 7 P < 0 01	16 4 ± 10 1
Dibenamine (1 10⁻⁴)	0 5	60	7	40 3 ± 11 1 P < 0 01	-11 6 ± 12 3
Dibenamine (5 10⁻⁵)	0.5	60	6	32 7 ± 12 5 P < 0 01	-26 0 ± 5 3 P < 0 01
Tolazoline (1 10⁻⁴)	0 5	120	7	91 6 ± 23 3 P < 0 01	-41 8 ± 31 1

or with depression of the carbohydrate metabolism by glycolysis inhibiting agents the contractile response was absent Stimulation of the lactic acid production coincided with contraction and ceased when the muscle had attained a certain degree of contraction Quantitative estimation of the lactic acid production indicated that contraction of vascular muscle required substantially greater energy than did the corresponding process in striated muscle This observation led to a study of the metabolism of phosphate compounds in vascular muscle during contraction by adrenaline whereby we found that the muscle quantitatively utilized those high energy phosphate compounds whose formation was assumed to result from stimulation of lactic acid production (LUNDHOLM and MOHME LUNDHOLM 1962 b)

The investigations pointed to a very close correlation between smooth muscle contraction and stimulation of the lactic acid production under anaerobic conditions and suggested that this stimulation may have been secondary to the contractile process — The present experiments indicate however that the two effects are dissociable Thus the lactic acid stimulation cannot be considered secondary to the contraction on the contrary the two reponses are assumed to be elicited by activation of separate mechanisms

Methods

In the experiments entailing coincidental determination of the lactic acid production bovine mesenteric arteries were used Approximately 15 min after slaughter an artery with a uniform diameter of 3 5—4 mm was dissected free and a segment removed and immersed in iced Tyrode solution About 60 min later the segment was opened lengthwise and specimens 10—12 mm long 15 mm wide and approximately 0 8 mm thick were prepared Following careful removal of the intima and drying with filter paper

l)th min) on tone and la tic ac d p oduct n f mesenteric a tery

D fferenc in lactic cid prod ction (mg/100 g)		Diff enc n ton (mm) at end of experiment		
Ad drug — Drug	D ff 1—3	Adr -Contr l	Drug Contr l	Adr drug — Drug
11 ± 36	12 5 ± 49 P < 0 0)	2 7 ± 0.59 P < 0 001	0)6 ± 0 79	0.20 ± 0 31
27.5 ± 6 8 P < 0 01	0 33 ± 3 7	1 6? ± 0.2) P < 0 001	0 91 ± 0.6 P < 0 01	0.50 ± 0.26
15 8 ± 6 3 P < 0 01	24) ± 7 4 P < 0 01	1 76 ± 0.26 P < 0 001	−0 51 ± 0.29	0 16 ± 0 24
0 9 ± 9.9	33 5 ± 12 8 P < 0 0)	3 41 ± 0 39 P < 0 001	0 04 ± 0.2?	0 0? ± 0.26
11 9 ± 14 6	79 8 ± 31 2 P < 0 0)	4 0 ± 0 45 P < 0 001	1 5 ± 0 36 P < 0 01	0 5 ± 0 2)

the p eparat ons were weighed and mounted n plastic frames whereby the load was evenly distributed ov the whole urfa e Changes of length in the direct on of the ci cular muscle fibres were ecorded by isotonic pen with a load of 10 g and a rat o of 1 10 The changes are her given in mm in relation to the initial lengths of the prepara t ons The organ baths conta ned 20 ml suspension solution at 38 C aerated with \ Glucose was added as indicat d in the text The lactic acid p oduct on was determined by measuring both the change n the lacti acid content of th muscl during the ex periment and the in eas of lactic acid content n the suspension solut on The lact c acid content was det rmined *ad modum* FRIEDEMANN and GRAESER (1933) with the mod fica tions reported by MOHME LUNDHOLM (1953) D fferences n lact c ac d p oduct on a e refe abl to pr parations f om the ame essel

Six per cent dext an (Pha ma a) was prepa ed f om sol d substan e with dist'll d water and had ac ord ng to the makers a colloid osmoti pressu of pproximat ly 30 mm Hg

The modes of p ocedur n th nd vidual runs of xp rim nts w e as follows

In th xperiments w th *ad n lyt* 1? pr pa ations er taken from th artery spec men 6 of them v p etr at d fo 30 min c d Tyrod olution onta n ng the relevant ad nol t drug T o untreated and tv o tr at d *prepa ations* ere then taken for l ct c ac d assa (n t al p pa at on th others wer mounted in o gan baths half of v h h onta n d an adrenolyt drug To tv o of the t eated and tv o of the untr ated p pa at ons d enal ne 2 10⁻⁶ as added e ery 30 min On t rminat on of the exper m nt the p parat ons and suspens on olut on e assayed fo lact acid

In th xperiments w th *d t an s* x p eparation mounted n glu ose fr e 6 per cent dextran olut on and anoth 6 in glu os f e Tyrode olut on Th ty minutes late t o p pa ations f om ch group of 6 e tak n fo d term nat on of the in tial lactic acid ont nt nd th usp ns on med um of th othe 4 as eplaced ith dextran or Tyrod s lut on onta n ng 0 5 per cent glucose E th adr nalin ? 10⁻⁶ o histam ine 5 10⁻⁶ as add d to t o of the 4 p parations in each group After the lapse of another 30 m n th p epa at on were moved fo assay

From the f rst ries w th *K Tyr d* t o p eparat on were taken for det rmination of the n t al la t a d ont t after wh ch four were mounted n glu ose f e K Ty odeand

four in glucose free Tyrode solution Two hours and four hours later the lactic acid production was determined in two of each group of 4 The preparations in the *second Tyrode series* were first mounted in glucose free Tyrode solution After 30 min two preparations were assayed for the initial lactic acid content, and the others solutions were changed to Tyrode containing 0 5 per cent glucose was added to 4 preparations and Tyrode solution with 0 5 per cent glucose to the other 8 To 4 of the latter adrenaline 2 10⁻⁴ was added every 30 min After 15 min half the preparations were removed for assay and after 120 min the remaining preparations were assayed

In experiments on *human umbilical artery* a segment of umbilical cord was immersed in iced Tyrode solution a few minutes after birth of the donor From this segment arterial specimens about 20 mm long were prepared and their longitudinal contractions recorded by isotonic pen with a load of 5 g The suspension medium was Tyrode solution containing 0 1 per cent glucose and aerated with 93 5 per cent O_2 and 6 5 per cent CO_2

Results

Effect of Adrenolytics on the Lactic Acid Stimulating and Contractile Actions of Adrenaline

The first series of experiments was designed to establish whether adrenolytics inhibited to an equal degree the contractile and the lactic acid stimulating effects of adrenaline The experiments were performed on mesenteric arteries under anaerobic conditions and the adrenolytics used were dihydroergotamine (DHE) dibenamine and tolazoline

For the opening run of experiments with DHE at a concentration of 1 10⁻⁴ glucose free Tyrode solution was used DHE *per se* stimulated the lactic acid production but had no demonstrable effect on the tone (Table I) It caused almost total inhibition of both the lactic acid stimulating and the contractile affects of adrenaline Since under the given experimental conditions the capacity of the muscle to form lactic acid may well have been limited by substrate deficiency experiments were also performed in Tyrode solution containing 0 5 per cent glucose. DHE again stimulated the lactic acid production but this time inhibited the tone The contractile effect of adrenaline was almost completely suppressed (fig 1B Table I) whereas its lactic acid stimulating effect was unchanged Adrenaline increased the lactic acid production by 27 9 ± 5 7 mg 100 g 120 min in the absence of DHE as compared to 27.5 ± 6 8 mg in the presence of DHE Under these conditions therefore DHE selectively inhibited the contractile effect of adrenaline without interfering with its stimulatory effect on lactic acid production

In experiments with 0 5 per cent glucose dibenamine at a concentration of 1 10⁻⁴ showed a somewhat similar behavior in that adrenline s contractil action was totally suppressed while its lactic acid stimulating effect was only partially blocked and remained statistically verifiable (Table I) — MOHME LUNDHOLM (1962 in experiments on isolated rabbit stomach had observed that dibenamine at a concentration of 1 10⁻⁴ changed the contractile effect of adrenaline into a relaxing action but failed to affect adrenaline s stimulation of lactic acid production — At the higher concentration of 5 10⁻⁴ dibenamine

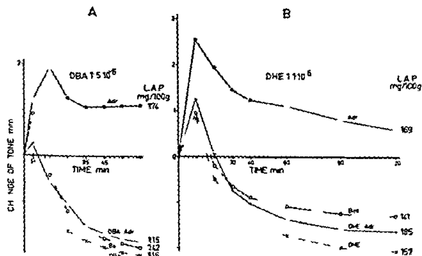


Fig 1 Eff ct f d h d oe got m (DHE) d diben mine DBA) on ton nd l ct d p oduct n (L A P l mes t art es n Tyrod sol t n th 0 5 per nt glu o e t d w th A Th p ep o p i at d in the ld f 30 min th DHE o DBA wh e af adren lin was dded

completely inhibited both the tone promoting and the lactic acid stimulating actions of adrenaline in addition to depressing the initial spontaneous increase of the muscle tone (Fig 1 A) Ten minutes after mounting the difference between the tone of the control and that of the dibenamine preparations was $1\ 17 \pm 0\ 37$ mm ($n = 12$ $p < 0\ 01$) The basal lactic acid production was also depressed by dibenamine in this series — an effect that was detectable even at the lower dibenamine concentration (Table I)

Tolazoline at a concentration of $1\ 10^{-4}$ practically suppressed the contractile and lactic acid stimulating actions of adrenaline The drug itself enhanced the vascular muscle tone (Table I)

Mechanism of Tonus and Lactic Acid Inhibiting Actions of Dibenamine

The inhibition of tone and the depression of lactic acid production by dibenamine at a concentration of $5\ 10^{-6}$ showed a quantitative relationship corresponding to that earlier demonstrated between lactic acid production and contraction (LUNDHOLM and MOHME LUNDHOLM 1962 a) These previous experiments had shown an average of 23 0 for the lactic acid production in mg per 100 g tissue per mm of contraction in the presence of 0 5 per cent glucose In the dibenamine experiments the lactic acid production decreased by 22 5 mg per 100 g per mm of postulated contraction Hence the effect of dibenamine on the lactic acid production is likely to have been due to blocking of the spontaneous muscle contraction

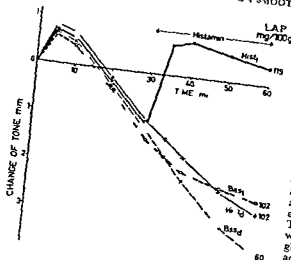
Bovine arteries contain approximately 1 0 μg noradrenaline per gram tissue (SCHMITERLOW 1948) The question thus arose whether the release of noradrenaline from the depots could have been responsible for the spontaneous augmentation of tone Had this been the case, dibenamine might well have exerted its effect by blocking noradrenaline Experiments were accordingly performed on human umbilical artery which has no sympathetic innervation and contains no noradrenaline (EULER 1946) Preparations from such artery underwent spontaneous contraction when transferred from iced Tyrode solution to the same solution at 38 C The effect therefore was probably attributable to temperature changes — a factor which may have an appreciable influence on the tone of isolated vascular preparations (BÜRGI 1944) Isolated umbilical artery also contracted on administration of the drugs tested in this and previous investigations (LUNDHOLM and MOHME LUNDHOLM 1962 a) i e adrenaline histamine acetylcholine barium ions and potassium ions It seems likely therefore that the relevant contracting and relaxing agents acted directly upon the muscle cells and not via liberation of noradrenaline from the mesenteric arteries

Dibenamine also inhibited the spontaneous contraction of isolated bovine tracheal muscle Since noradrenaline relaxed this tissue it was probable that dibenamine at the higher concentrations had had a more general spasmolytic action upon tone increasing stimuli FURCHGOTT (1954) too has demonstrated such an action

Effects of Adrenaline and Histamine upon Lactic Acid Production in Dextran Solution under Anaerobic Conditions

BOZLER (1959) observed that smooth muscle rapidly lost its responsivity in pure saccharose solution unless calcium ions were present We studied the effects of adrenaline and histamine on the lactic acid production and tone of vascular muscle in a suspension solution of 6 per cent dextran in N_2 Although this solution was hypotonic in relation to the muscle the latter s weight did not increase when it was transferred from the iced Tyrode solution to the dextran solution After 60 min in oxygenated glucose free dextran solution at 38 C the weight had decreased by an average of 11 per cent ($n = 6$) as compared to a corresponding value of 22 per cent in Tyrode solution at 38 C Thus the weight reduction was less in dextran than in Tyrode solution but was remarkably small These results like those of BOZLER and LAVINE (1958) suggest that the electrolyte osmotic pressure is not the only factor governing the water content of smooth muscle

In dextran solution one run of experiments was conducted with adrenaline and one with histamine Concurrent experiments in Tyrode solution served as controls The results for tonus are shown in Fig 2 (histamine experiments) and for lactic acid production in Table II



F g 2 Eff ct of histamine on tone and lact c acid p oduct on (L.A.P.) of mesent arteries in Tyrod (t) and dextran (d) solut on The prep-ar t ons w e first suspended in el ose f e so utions f r 30 min. The sol t ons ere then epla ed with oth rs containing 0 5 per ent glu ose and h t mine 5 10⁻⁵ was added

Tabl II Effe t f ad enaline and histamine on th lact c acid pr du tio and t ne f mesenteric a teries in Tyrod and d xt an lution with 0 5 per cent gluco Lactic acid product on (L.A P) in mg per 100 g per 30 min

	Tyrod sol t n			Dextran sol t on		
	Contr l L A P 30—60 min	Increase of L.A.P afte drug 30—60 min	Chang of tone mm, 10 min after drug	Control L.A P 30—60 min	Increase of L A.P fter drug 30—60 min	Chang of t ne mm 10 min after drug
Adrenal n (n = 8)	67 3 ± 10.2	21 7 ± 5 8 P < 0 01	2 33 ± 0 35 P < 0 001	43 6 ± 3 8	15 5 ± 6 0 P 0 0	-0 66 ± 0 07 P < 0 001
Histamin (n = 6)	106 4 ± 10 1	17 8 ± 6 44 P < 0 0	1 81 ± 0.28 P < 0 001	60 0 ± 7 4	4 1 ± 16 5 P < 0 05	0 70 ± 0 06 P < 0 001

D fferenc L.A P contr l Ty ode — nt ol dextran = 30 8 ± 7 7
P < 0 001

The spontaneous tone fell towards the end of the experiment som what faster in dextran than in Tyrode solution Histamine and adrenaline produced marked contraction in Tyrode solution but none whatsoever in dextran solution though they evidenced a certain effect by retarding somewhat the spontaneous decrease of tone After 15 min the tonus depression was 0.25 ± 0.09 mm ($p < 0.05$) less in the histamine experiments than in the adrenaline experiments than in the control ($p < 0.05$)

Table III Effect of Na Tyrode and K Tyrode on lactic acid prod tion (mg per 100 g) of mesenteric arteries Tyrode without glucose

Na Tyrode		K Tyrode	
0—120 min	0—240 min	0—120 min	0—240 min
51 4 ± 4 4	54 8 ± 11 0	54 4 ± 5 9	59 0 ± 9 4

D fference K Tyrode — Na Tyrode 0—120 m n = 3 1 ± 4 5
K Tyrod — Na Tyrode 120—240 min = 1 2 ± 12 9

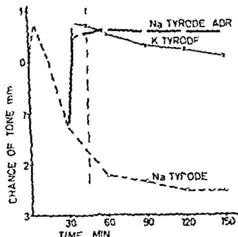
The control preparations had in dextran solution a lactic acid production averaging ($n = 14$) 63 per cent of that recorded in Tyrode solution the reduction was statistically significant (Table II) Both adren line and histamine however appreciably stimulated the lactic acid production in dextran solution Histamine had a somewhat greater effect in dextran than in Tyrode solution the pertinent difference amounting to 29 3 ± 17 8 mg/100 g/30 min which was not statistically significant It was evident, however that the stimulation of lactic acid production after adrenaline and histamine persisted even though the contractile effects were blocked

Effect of Potassium Ions upon Tone and Lactic Acid Production

With a slight increase of 4—8 meq in the potassium content of the Tyrode solution there was no effect on the tone or the lactic acid production under anaerobic conditions Under aerobic conditions the spontaneous elevation of tone was inhibited Not until one fourth to one half of the sodium ions in the Tyrode solution had been exchanged for potassium ions did the preparations show a contractile response under anaeraboic conditions To ensure a maximal effect of potassium we used a Tyrode solution in which all sodium ions had been replaced with potassium ions The solutions will be subsequently referred to as K Tyrode and Na Tyrode

Glucose free Na Tyrode and K Tyrode were used for the first run and the preparations were immersed in the respective solutions at the very outset of the experiment The lactic acid production was determined after 2 and 4 hours The tone reached a maximum after 10 min in the K Tyrode experi ments and by that time was 2 2 ± 0 23 mm ($p < 0\ 001$) higher on the average ($n = 11$) than the corresponding figure in the Na Tyrode experiments After 120 min the tone curves crossed and after 240 min the tone level was 1 3 ± 0 25 mm ($p < 0\ 001$) lower in K Tyrode than in Na Tyrode No difference in lactic acid production was demonstrable between Na Tyrode and K Tyrode after 2 or 4 hours (Table III) A possible explanation was that the muscle had fully utilized its carbohydrate reserves during the first two hours

Fg 3 Effect of K Tyrod and ad enaline n t e of mesenteric arteries All pr p- ar t o were susp d d n glucose f ee sol t on of K Tyrode for th first 30 mi Th sol tions were then x hanged for Na Tyrod with 0 5 pe cent gl cose (control) o the ame with 2 10⁻⁸ d na l e added or K Tyrode w th 0 5 per ent gl ose Th la t ac d p oduction was de erm n d f r the periods 30—45 m n and 45—150 mi



Tabl II Effe t of K Tyrode a d ad enaline on to and la tic acid production (L A P) f me enteric arteries The fir t period 0—30 min without gluc se The second period 30—150 min with 0.5 per cent glucose K Tyrode or adrenal ne was added at 30 Number of tests = 9

	Cha g f tone mm b tween 30—45 min	La t c acid prod ct on (mg per 100 g)	
		Du ing contr ction 30—45 min	After c ntraction 45—1 0 min
Contr l	−0 6? ± 0 07 P < 0 001	46.2 ± 12 6	?57.5 ± ?7.5
K Tyrode	2 11 ± 0 34 P < 0 001	20 8 ± 9 7	246 7 ± 10.9
Ad en l ne	1.84 ± 0.29 P < 0 001	61 1 ± 11 7	260.2 ± 29.2

D fference L.A.P Adrenalin — cont ol = 14 9 ± 3 6 (P < 0 0)
K Tyrod — ontrol = −2 3 ± 4 5 (P ~ 0 01)

In the next run therefore we studied the lacti acid production in the presence of 0 5 per cent glucose both during the contraction phase following addition of K Tyrode and when the tone had reached a constant level A comparative series vas run with adrenaline In the K Tyrode experiments the tone as shown in Fig 3 reached a maximum in approximately five minutes and remained at about that level until the end of the experiment Approximately the same tone level was attained after addition of adrenaline It was found in other experiments with K Tyrode that in the presence of glucose no depression of tone occurred after 4 hours The relaxation manifest after two hours in the preceding K Tyrode experiments did not therefore

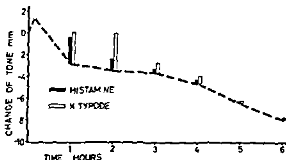


Fig 4 Stimulating effect of K Tyrode and histamine ($5 \cdot 10^{-6}$) on mesenteric artery after varying periods of substrate depletion and anaerobic conditions Each point is the mean of three experiments on different preparations

represent a toxic effect on the contractile mechanism but was probably a result of substrate deficiency The effect on the lactic acid production was interesting (Table IV) During the period from 30 to 45 min in the adrenaline experiments the usual stimulation of lactic acid production as compared to the controls was observed In the experiments with K Tyrode on the other hand the lactic acid production was inhibited to the extent of 25 3 ± 4 5 mg/100 g/15 min ($p < 0.01$) as compared to the controls During the period from 15 to 120 min however the lactic acid production values showed no significant difference either in the control adrenaline or in the K Tyrode experiments In K Tyrode the lactic acid production was thus inhibited during the contraction phase but was thereafter unaffected Under these conditions therefore there was apparently a selective stimulation of the contractile process without coincident activation of the carbohydrate metabolism

Contractile Effects of Various Drugs with Increasing Substrate Depletion

Several different interpretations could doubtless be placed upon the fact that potassium ions selectively stimulated the contractile process of smooth muscle On the theory that in vascular muscle the energy requirements for the contractile process were relatively great — as indicated by the results of other experiments — it was plausible to assume that potassium ions were more conducive than other tone promoting agents to the muscle's utilization of its preformed energy reserves This hypothesis was tested by comparative studies of the maximal contractile effects of various drugs with respect to varying degrees of substrate depletion The contractile effect of adrenaline was abolished by storage of the arterial preparation for approximately 60 min in glucose free Tyrode solution under anaerobic conditions whereas the effect of histamine persisted longer (LUNDHOLM and MOHME LUNDHOLM 1962 a) The contractile effect of potassium ions however was influenced even less than that of histamine by substrate depletion Fig 4 illustrates the maximum contraction level reached by preparations from the same vessel on treatment with histamine $5 \cdot 10^{-6}$ or K Tyrode after storage for various periods in glucose free Tyrode solution under anaerobic conditions A tenfold increase of the histamine dose failed to augment the contractile response An interesting observation is that

the tone produced by K Tyrode did not remain at the elevated level but fell after 15—30 min to a lower level than that associated with histamine Since the K Tyrode on addition of glucose had the capacity to elicit a substantial increase of tone even after 4—5 hours the attenuation of the contractile effect could not have been caused by damage to the contractile mechanism

When the preparations were treated with dinitrophenol under aerobic conditions the behavior was similar Here too the contractile effect was greater and of longer duration after K Tyrode than after adrenaline histamine or barium ions

Discussion

According to the classical conception of the relationship between muscle contraction and stimulation of the metabolism the metabolic increase is secondary to the contraction and is induced via the augmentation of ADP and/or inorganic phosphate which is thought to accompany contraction This view is founded on studies of striated muscle Other investigations of striped muscle have shown however that under certain experimental conditions selective stimulation of the metabolism may occur without activation of the contractile mechanism (WILKIE 1954)

We are *a priori* inclined to assume that the increased lactic acid production caused by tone promoting drugs in experiments on smooth muscle was likewise secondary to the contraction — the more so in that the latter was distinctly correlated quantitatively and temporally to the lactic acid production (LUNDHOLM and MOHME LUNDHOLM 1962 a) The experiments in dextran solution as well as the reported studies on the effect of DHE upon the contracting and lactic acid production stimulating actions of adrenaline demonstrated however that it was possible to block the contractile effects of tone promoting drugs without influencing their stimulation of lactic acid production The experiments with potassium on the other hand showed that a certain degree of contraction could occur without stimulation of the metabolism The most plausible explanation was that the metabolism and the contractile process were stimulated via different mechanisms This hypothesis was supported by the observed stimulation of the carbohydrate metabolism in smooth muscle relaxed by adrenaline (MOHME LUNDHOLM 1953 1960 1962)

In our view therefore a drug such as adrenaline may stimulate in smooth muscle either the contractile process as well as the carbohydrate metabolism or the carbohydrate metabolism alone In the former instance contraction is elicited and the muscle utilizes the high energy phosphate compounds associated with the augmentation of lactic acid production (LUNDHOLM and MOHME LUNDHOLM 1962 b) If the initial muscle tone is high and only the lactic acid production is stimulated relaxation will result Adrenaline reversal in isolated smooth muscle can probably be explained in this way (MOHME LUNDHOLM 1962)

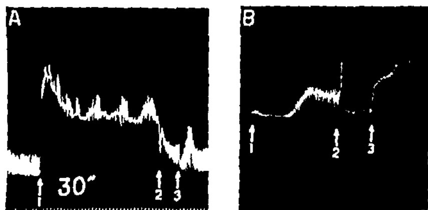


Fig. 5. Rabbit jejunum.

A. Tyrode solution with 0.1 per cent glucose aerated with 93.5 O + 6.5 CO. At the first arrow carbacholine 2.5 · 10 (2) adrenaline 1 · 10 (3) adrenaline 5 · 10

B. The preparation had been suspended for 2 hours in glucose free Tyrode solution a rat d with N. At the first arrow N was exchanged for O (2) carbacholine 2.5 · 10 (3) adrenaline 1 · 10

It could naturally be asked whether the stimulated metabolism was not the primary response and the muscle contraction a result of the increased supply of high energy phosphate compounds. To this it could be replied that although glucose in experiments on substrate depleted mesenteric arteries increased the lactic acid production and the high energy phosphate compound content to a greater extent than did adrenaline the muscle failed to contract (LUNDHOLM and MOHME LUNDHOLM 1962 b). The experiments with potassium ions in which the lactic acid production was inhibited despite contraction of the muscle also implied that the primary cause of the smooth muscle contraction was not to be found in an increased supply of high energy phosphate compounds but rather in the muscle's capacity to utilize its existing energy reserves.

The intensified carbohydrate metabolism and the synthesis of high energy phosphate compounds could however — at least under certain experimental conditions — be of fundamental significance for the contractile effect exemplified in the experiment in Fig. 5. In normal rabbit gut carbacholine had a tone promoting effect to which the relaxing action of adrenaline was antagonistic. Following storage for two hours in glucose free Tyrode solution under anaerobic conditions and subsequent transferal of the preparations to an aerobic environment the contractile effect of carbacholine was observed to be of very short duration. Adrenaline which when administered alone tended to relax intestinal preparations here potentiated the stimulatory effect of carbacholine. Similar experimental results have been reported by FURCHGOTT (1950) and AXELSON, BUEDING and BÜLBRING (1959). Furthermore MOHME LUNDHOLM (1960) has shown that adrenaline stimulates glycogenolysis and lactic acid production even in substrate depleted gut. It seems probable that

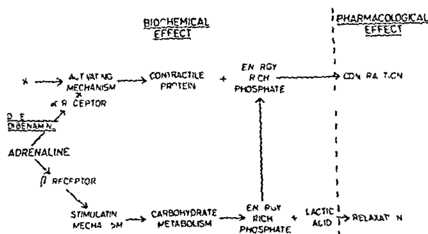


Fig 6 Showing the probable relation ship between the biochemical and pharmacologic effects of adrenaline in smooth muscle At high concentration both DHE and dibenamin may block the stimulation of lactic acid production

in the experiment described above carbacholine activated the contractile mechanism while adrenaline stimulated the carbohydrate metabolism and the energy production Only when both these mechanisms had been stimulated did the muscle tonus reach an elevated level

The contractile process in vascular smooth muscle apparently requires an abundant supply of energy In an earlier investigation (LUNDHOLM and MOHME LUNDHOLM 1962 a) we estimated that the muscle s known preformed high energy phosphate compounds suffice only for about 10 per cent of a total contraction It would thus seem that the contractile process of vascular muscle depends far more than does that of striated muscle upon a continuous energy production In contrast to striated muscle moreover smooth muscle — at least that from the mesenteric artery — is unlikely to have a resting state for only with total suppression of the metabolism or with extreme substrate depletion was it possible to induce complete relaxation of vascular muscle Under basal conditions the muscle invariably possessed a certain amount of tone i e a certain degree of contraction (LUNDHOLM and MOHME LUNDHOLM 1960) Tonus changes of smooth muscle therefore apparently reflect varying degrees of activity rather than transition from a resting to an active state It follows that no specific resting metabolism occurs in vascular muscle but that even the basal energy production is utilized to some extent by the muscle for maintenance of a certain level of activity Herein may lie the possible significance of coincident stimulation of the contractile mechanism and the metabolism

Our conception of the relationship between the biochemical and pharmacologic responses of smooth muscle to adrenaline — the drug we have thus far studied most extensively — is illustrated in Fig 6 When adrenaline stimulates

both the contractile mechanism and the carbohydrate metabolism the con tractile effect predominates and the tone increases Selective stimulation of the carbohydrate metabolism as in intestine bronchial muscle etc or selective blocking of the contractile effects of adrenolytics is followed by re laxation which results from the increase of lactic acid in the muscle (MOHME LUNDHOLM 19o3 1962) Even when the contractile mechanism is stimulated however the concomitant lactic acid production may have an inhibitory effect for the stimulatory effect of adrenaline on smooth muscle is enhanced in alkaline and attenuated in acid solution (ALPERN 1924 TOBIAN MARTIN and EILERS 1959) In Fig 6 we have tentatively identified adrenaline s stimulation of the contractile mechanism as an action upon *alpha* receptors (AHLQUIST 1948) and the lactic acid stimulating relaxing effect as an action upon *beta* receptors Whether or not this interpretation is justified must remain at present open to question since *alpha* and *beta* receptors are not confined to smooth muscle and their significance in other tissues has not yet been fully elucidated

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From the Departm nt of Pharmacology Lniversity of Goteborg S eden

Contraction and Glycogenolysis of Smooth Muscle

B

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R ed 21 June 19

Abstract

LUNDHOL L and E MOHME LUNDHOLM *Contract on and gly o-enolysis of mooth muscle* Acta physiol scand 1963 57 125—129 — The question as to hether smooth muscle contraction is accompan ed by nten ified glycogenolysi was studied in experiments on rabbit gut on bovine tracheal muscle and on mesenteric artery Under aerob c cond t ons electr cal stimulat on augm nted the glycogenol s s in these types of smooth muscle Of tonus increas ng drugs only c ba hol ne enhanced glycogen breakdown n tracheal mus le Und r anae ob c conditions contraction of smooth mus le as not accompanied by gl ogenolysis desp te the fact that l ctic ac d p o-duct on as ncreased Smooth mu le contra tion the efore was not onsistently associated th increased gl ogen breakdo n In this respect ther seems to b a d ff rence bet n smooth muscle an l stri ted muscle

Contraction of striated muscle is accompanied under both aerobic and anaerobic conditions by substantial glycogenolysis (CORI 1956) In recent personal experiments contraction of smooth muscle under anaerobic conditions as found to be assoc ated ith parallel stimulation of lactic acid production (LUNDHOLM and MOHME LUNDHOLM 1962) In view of these observations it was of interest to ascertain hether increased glycog nolysis as also a concomitant of smooth muscle contraction and to determine the extent to hich stimulation of lactic acid production as associated ith intensification of glycogenolysis

We found how ever that stimulation of glycogenolysis as not a consistent concomitant of smooth muscle contraction It thus respect smooth muscle and striated muscle apparently diff r

Table I Effect of ele trical stimulation and contracting d ugs on glycogenolysis of rabbit inte t e bo n t acheal muscle and mesenteric artery Glycogen content in mg per cent P = probab l ty th t the effect was d e to chan e

Smooth muscle organ	No of test	St mulation	Time min	Ba al glycogen content	Cha ge of glycogen content
Rabb t intestine	14	lectrical 4 mA, 10/sec	3	114 ± 7 7	− 6 1 ± 1.8 P < 0 01
	12	carbachol n 5 10⁻⁷	10	75 ± 1 9	+ 1 8 ± 3 0
Bovine tracheal muscl	1	electr cal 6 mA, 10/sec	4	251 ± 29 (	−33 3 ± 10.3 P < 0 05
	15	carbachol n 2 5 10⁻⁷	20	320 ± 31.5	−19 3 ± 7.8 P < 0 05
Bov e mesenteric artery	15	elect cal 3 mA, 10/sec	3	161 ± 20.5	−67 1 ± 9.4 P < 0 001
	21	ad enaline 1 10⁻⁶	5	182 ± 18.3	+11 9 ± 4 3 P < 0 0

Methods

The experiments were performed on rabbit gut and on bovine trach al muscl and mesenteric artery The bovine pecimens were obtai ed from abattoir material abo t 15 min after slaughter and immersed n ced Tyrode solution They were then cut into pieces weighing 0 2 — 0 4 g wh ch were mounted in organ baths conta nin 20 ml Tyrode solution at 38 C and aerated with 93 5 per cent O and 6 5 per cent CO_2 or N Unless otherw ise stated the olution was glucose free The contractions were r corded by isotonic pen hich had a load of 5 g for rabb t intestin 40 g for tracheal muscle and 10 g for mesenteric artery

In the experiments with electri al stimulation under aerob c cond tions the Tyrode solution was removed f om the control and test preparations after about 20 min and the preparations were then stimulated, via platinum hooks with condenser discharges of an intensity suff cient to produce maximal contractile reponses (3—6 mA 10 per sec Unt l th Tyrode solution was removed the impulses generated by the stimulator were too weak to produce maximal cont action When the control and test preparations were fully contracted after a period of 3—4 m n they were assayed for glycogen In the experiments th drugs under aerobic cond t o is the relevant agents were added to the baths containin the test preparations and the latter were removed for assay when they had reached a stat of maximal contra t on

In some experiments the lact ac d production was also studied and here the init al glycogen and lactic acid levels were determined in the preparations at the start of the experiment Approximat ly one third of th p eparations were taken for glycogen assa On termination of th experiment the glycogen and lact c a id contents w re again determined in th preparat ons and the increase of lact c acid was measured the suspens on medium For calculat on of th lactic acid product on the amount of lactic acid in the muscl at th start of the experiment was subtracted from the total amount in the muscle a d th uspension medium at th e end of the experiment. Electrical

Table II. The effect of electrical stimulation, adrenaline and mono-iodoacetic acid on glycogenolysis and lactic production of mesenteric artery. Glycogenolysis and lactic acid product in mg/100g/[illegible]0 min. N = number of tests. *P* = probability that the effect was due to chance

	Electric stimulation anaerobic		Adrenaline		Mono-iodoacetic acid
	0 glucose n = 6	0.5 glucose n = 6	anaerobic n = 9	aerobic n = 6	anaerobic n = 6
Initial glycogen mg per cent	115 ± [illegible]0.1	115 ± 20.1	[illegible]00 ± 29.1	[illegible]45 ± 33.7	190 ± 31.7
Glycogenolysis, control	36.7 ± 1[illegible].3	[illegible]1 ± 16.5	[illegible]6.9 ± 1[illegible].4	34.6 ± 15.8	1[illegible]0.3 ± 14.9
Glycogenolysis, test	33.5 ± 14.9	− [illegible]0.7 ± 14.5	72.3 ± 14.8	−0.8 ± 16.0	104.3 ± 14.0
Difference glycogenolysis, test-control	−3.1 ± 3.9	−2[illegible].8 ± 17.1	−4.3 ± 7.5	−3[illegible].4 ± 10.[illegible] P < 0.0[illegible]	−16.0 ± 10.7
Lactic acid production, control	81.1 ± 12.8	158.8 ± 12.8	41.0 ± 8.9	6.7 ± 2.8	48.9 ± 8.7
Lactic acid production, test	95.8 ± 13.0	171.2 ± 11.[illegible]	58.3 ± 13.9	10.4 ± 4.0 P < 0.05	20.3 ± 6.4
Difference lactic acid production test-control	14.7 ± 5.7 P < 0.05	15.8 ± 13.1	17.3 ± 6.2 P [illegible] 0.0[illegible]	3.7 ± 4.3	−[illegible]6 ± 11.5 P < 0.05

stimulation under anaerobic conditions was produced in Tyrod solution with period[illegible] A C [illegible] 15 V 50 cps. It [illegible] that under these circumstances the bulk of the current passed through the Tyrod solution.

In the experiments with mono-iodoacetic acid the latter was added at the very outset of the experiment in a concentration of 0.0[illegible] M neutralized with NaOH to pH 7.4. In the long term experiments with adrenaline the latter was added in a concentration of 1 : 10[illegible] after 1, 15, 30 and 4[illegible] min.

The glycogen content was determined by a previously reported method (LUNDHOLM and MOHME-LUNDHOLM 1957) and the lactic acid content *ad modum* FRIEDEMANN and GRAESER (1933) with certain modifications (MOHME-LUNDHOLM 19[illegible]3).

Results

Aerobic Conditions. — When subjected to electrical stimulation for 3—4 minutes rabbit gut as well as bovine tracheal muscle and bovine mesenteric artery showed appreciable glycogenolysis and concomitant contraction (Table I). The effect was greatest in arterial muscle and relatively small in rabbit intestine — possibly because only about 20 per cent of the latter consisted of smooth muscle.

Drug-elicited contraction yielded divergent results (Table I). Only *in the* experiments with tracheal muscle was glycogenolysis demonstrable despite the fact that the contractile effect was as pronounced as that observed after electrical stimulation. In the rabbit gut experiments carbacholine elevated the lactic acid content of the preparations by 10.[illegible] ± 3.1 mg per cent ($n = 7$, $p < 0.01$) but did not stimulate glycogenolysis. Adrenaline despite its

contractile effect inhibited the glycogenolysis in mesenteric artery This effect was confirmed in another series of long term experiments (Table II) in which the lactic acid production was also measured Here adrenaline notwithstanding marked contraction of the muscle totally inhibited the spontaneous glycogenolysis while concomitantly the lactic acid production increased by 10 4 ± 4 0 mg/100 g/60 min (p < 0 05)

Anaerobic Conditions — Under anaerobic conditions electrical stimulation of mesenteric artery though augmenting the lactid acid production failed to increase the glycogenolysis (Table II) Since a possible explanation was that glycogenolysis had already been maximal under anaerobic conditions experiments were performed in the presence of 0 5 per cent glucose The spontaneous glycogenolysis in the control specimens (Table II) was inhibited by as much as 14 5 ± 5 2 mg/100 g/70 min ($p < 0\ 05$) Under similar experimental conditions we had observed aerobic synthesis of glycogen (LUNDHOLM and MOHME LUNDHOLM 1963) Not even in the presence of glucose however did electrical stimulation intensify glycogenolysis the glycogen content of the preparations remained almost constant Nor under anaerobic conditions did the antiglycolytic effect of adrenaline emerge

It is clear from Table II that the basal glycogenolysis and the lactic acid production were by no means commensurate In the control experiments with adrenaline under anaerobic conditions the glycogen breakdown was almost twice as great as the lactic acid production whereas in the experiments with electrical stimulation in the absence of glucose it was only half as great This appreciable discrepancy may well have been due to seasonal factors — a phenomenon which has been discussed in an earlier paper (LUNDHOLM and MOHME LUNDHOLM 1960)

With mono iodoacetic acid there was a manifest disparity between glycogenolysis and lactic acid production the latter was reduced by 58 per cent but the former by only 13 per cent and the effect on glycogenolysis was not statistically verifiable (Table II)

The augmentation of lactic acid production to which adrenaline and electrical stimulation gave rise under anaerobic conditions was not accompanied by increased glycogen breakdown

Discussion

In these experiments contraction of smooth muscle was not consistently attended by intensification of glycogenolysis The correlation between lactic acid stimulation and muscle contraction was far more evident than that between glycogenolysis and contraction Glycogenolysis and lactic acid production were not correlated On the contrary substantial amounts of lactic acid were sometimes formed from intermediate products of the carbohydrate metabolism — In mesenteric artery these intermediate products may — in the absence of

glucose — form approximately 50 mg lactic acid per 100 g tissue (LUNDHOLM and MOHME LUNDHOLM 1960)

It may be of interest in this context to compare the maximum velocity of glycogenolysis and the lactic acid production in mesenteric artery On electrical stimulation under aerobic conditions glycogen breakdown averaged 20 mg/100 g/min while the lactic acid production under anaerobic conditions and in the presence of 0 5 per cent glucose was 2 5 mg/100 g/min It is evident that in these circumstances the lactic acid production rate could not have been governed by the glycogenolytic reaction but that other reactions limiting the velocity were of greater significance — In the case of advanced substrate depletion however a closer correlation between lactic acid production and glycogenolysis had been observed (MOHME LUNDHOLM 1960)

In smooth muscle relaxed by adrenaline a glycogenolytic effect was noted (LUNDHOLM and MOHME LUNDHOLM 1957) whereas the contractile effect of adrenaline was apparently associated with inhibition of glycogenolysis The behavior of the phosphorylase activity on addition of adrenaline also varied in tracheal muscle which was relaxed by adrenaline the activity increased whereas in mesenteric artery it was not demonstrably affected (MOHME LUNDHOLM 1962)

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Effect of Insulin on the Carbohydrate Metabolism of Smooth Muscle

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Abstract

LUNDHOLM L and E MOHME LUNDHOLM *Effect of insulin on the arbohyd ate metabolism of smooth muscle* Acta physiol scand 1963 *57 130—134* — *In experiments on isolated stomach muscle from rabbit in* Krebs Henseleit bicarbonate buffer and on bovine mesenteric arteries in Tyrode solution insulin stimulated the synthesis of glycogen but had no demonstrable effect on the lactic acid production

Insulin has a well documented effect on the carbohydrate metabolism of striated muscle (for review *vide* STADIE 1954 RUSSEL and WILHELMI 1960) and is also reported to stimulate glucose utilization in heart muscle (BLEEHEN and FISHER 1954) The question whether insulin affects the carbohydrate metabolism of smooth muscle however apparently has not been resolved Since late complications of diabetes involve the vascular system to a high degree it is of particular interest to establish whether or not insulin influences the metabolism of vascular muscle

In bicarbonate buffer insulin was observed to stimulate the synthesis of glycogen and the lactic acid production in isolated rat diaphragm whereas in phosphate buffer only the glycogen synthesis was stimulated (SHAW and STADIE 1957) The first run of experiments on rabbit stomach muscle was therefore performed in Krebs Henseleit bicarbonate buffer at pH 7 4 The relatively high basal lactic acid production in this slightly alkaline solution was thought however to reduce the possibilities of demonstrating any stimulating effect of insulin on that production For this reason the second run of experiments on bovine mesenteric arteries was done in Tyrode solution at pH 7 15 in which medium the spontaneous lactic acid production was diminished The lactic

acid production of muscle appears to be in some degree a function of the pH of the suspension solution (KERLY and RONZANI 1933)

Insulin stimulated the synthesis of glycogen both in rabbit stomach muscle and in bovine mesenteric arteries but did not affect the lactic acid production

Methods

Isolated Rabbit Stomach

Rabbits weighing 2—3 kg were killed by a blow on the head Their stomachs were removed opened along the lesser curvature and flushed with Krebs Henseleit bicarbonat buffer of room temperature The muscle coat was carefully divested of mucosa and connective tissue Specimens 15 mm wide 20—25 mm long 0 8—1 0 mm thick, and 0 3—0 4 grams in weight were prepared. Each preparation was mounted along the edges in a small plastic frame which ensured an even distribution of the load The elongation or contraction in the direction of the circular muscle fibres was recorded by isotonic pen with a ratio of 1 10 and a load of 5 g The muscle coat was thickest along the less curvature and the thickness also increased towards the pylorus To secure the greatest possible uniformity of thickness two contiguous portions from each specimen were used as test and control preparation respectively since in general the lactic acid content was higher and the lactic acid production per gram greater in the thicker preparations.

Six preparations were taken from each stomach Two of them were immediately assayed for glycogen and lactic acid and the remaining four were mounted in organ baths containing 10 ml Krebs-Henseleit bicarbonate buffer at 37 C and with the following composition (in M/1) 0 12 NaCl 0 0047 KCl 0 0025 CaCl 0 0012 MgSO 0 0012 KH PO_4 0 025 NaHCO and 0 2 per cent glucose Aerated with 95 per cent O_2 and 5 per cent CO the solution had a pH of 7 4 For the experiments with insulin the solution contained 0 1 I U crystalline insulin (Vitrum) per ml.

Bovine Mesenteric Arteries

A segment of mesenteric artery with a uniform diameter of 3 5—4 mm was removed approximately 15 min after slaughter and immersed for 60 min in iced Tyrode solution The adventitia was then carefully dissected away and the specimen cut open lengthwise. From each arterial segment six specimens with a width of 15 mm a length of 10—12 mm and a thickness of approximately 0 6 mm were prepared They were mounted in plastic frames as above and transferred to organ baths containing 10 ml Tyrode solution with the following composition (in M/1) 0 138 NaCl 0 003 KCl 0 0018 CaCl 0 001 MgCl 0 012 NaHCO 0 036 NaH PO_4 and 0 2 per cent glucose. Aerated with 93 5 per cent O_2 and 6 5 per cent CO_2 the solution had a pH of 7 15 In the experiments with insulin the latter concentration was 0 1 I U per ml The preparations were subjected to a load of 10 grams

After two hours control and insulin preparations were quickly frozen with difluorodichloromethane (frigen 12) containing carbon dioxide snow (— 75 C) Approximately one third was taken for glycogen assay as described in an earlier paper (LUNDHOLM & MOHME LUNDHOLM 1957) The remainder was assayed for lactic acid with L (+) lactic acid dehydrogenase *ad mod* in PFLEIDERER and DOSE (1955) with the modification of SCHOLZ et al (1959) as previously described (MOHME LUNDHOLM 1962) The suspension medium was deproteinized by addition of 3 per cent perchloric acid to give a pH of 4 6 boiling and centrifugation The lactic acid content of the suspension solution was determined in 1 6 ml after 0 5 ml buffer at a concentration (2 0 M) four times higher than that previously used had been added to the suspension solution.

Table I The effect of insuline on glycogen content of rabbit stomach muscle and bovine mesenteric artery Glycogen content in mg per cent

Preparation	Initial value	Control after 120 min	Insuline after 120 min
Rabbit stomach muscle (n = 11)	75.4 ± 6.6	74.7 ± 5.5	99.7 ± 10.1
	Difference between	Control—Initial =	−0.7 ± 4.9
		Insuline—Initial =	24.5 ± 10.6 P < 0.05
		Insuline—Control =	25.0 ± 8.4 P < 0.02
Bovine mesenteric artery (n = 8)	175.7 ± 19.0	223.5 ± 14.2	245.8 ± 14.4
	Difference between	Control—Initial =	47.8 ± 20.1 P < 0.05
		Insuline—Initial =	70.1 ± 22.6 P < 0.02
		Insuline—Control =	22.2 ± 8.6 P < 0.05

n = number of tests P = probability that the effect was due to chance.

The changes in glycogen content were determined directly in the preparations For calculation of the lactic acid production the amount in the preparations at the start of the experiment was subtracted from the total amount in preparations and solution at the end of the experiment

Results

I Isolated Stomach Muscle from Rabbit

Tone The preparations consistently relaxed during the first 30—60 min of the experiment Subsequently approximately one half of them showed a spontaneous elevation of tone which sometimes reached a level well above the initial value No effect on tone that could have been attributed to insulin was observed

Glycogen Content The effect on the glycogen content is demonstrated in Table I During the 120 minute period in the glucose containing suspension medium the glycogen content of the control preparations remained at its initial level In 10 of the 11 experiments with insulin the glycogen content increased by an average of one third in relation to the basal values and to the glycogen content of the control preparations The increase was statistically verifiable Apparently therefore insulin stimulated the synthesis of glycogen in smooth muscle

Table II The effect of insuline on lactic acid production of rabbit stomach muscle and bovine mesenteric artery Lactic acid production in mg/100 g/120 min

Preparation	Lactic acid production	
	Control	Insuline
Rabbit stomach muscle (n = 14)	76 3 ± 16 4	60.2 ± 11.2
	Difference Insuline—Control = —16 0 ± 17 4	
Bovine mesenteric artery (n = 8)	—24 6 ± 7 6	—21 4 ± 71 0
Initial lactic acid content 73 8 ± 6 6 mg /	Difference Insuline—Control = 3.3 ± 4 9	

Lactic Acid Production Insulin had no appreciable effect on the lactic acid production of stomach muscle The control preparations had a strikingly high lactic acid production but it was not demonstrably influenced by insulin

II *Bovine Mesenteric Arteries*

Tone Mesenteric artery preparations showed an initial increase of tone which reached a maximum after about 10 min and was followed by gradual relaxation Insulin did not appreciably affect the tonus curves

Glycogen Content Even in the absence of insulin the glycogen content rose in seven of eight control experiments by an average of 70 1 ± 22 6 mg/100 g/120 min ($p < 0.02$) — see Table I This finding corroborates SCHILLER's (1950) observation that in the presence of glucose *in vitro* arterial tissue may synthesize glycogen Addition of insulin reinforced the glycogen synthesizing capacity of the arterial muscle In 7 of 8 insulin experiments the synthesis of glycogen increased and the increase was on the average 22 2 ± 8 6 mg/100 g/120 min ($p < 0.05$) greater than that observed in the control experiments

Lactic Acid Production In six of eight experiments the preformed lactic acid was metabolized so that the lactic acid production was on the average negative (Table II) Insulin had no detectable effect on the lactic acid metabolism the value for lactic acid disappearance accorded with that in the control experiments

Discussion

Insulin stimulated the synthesis of glycogen in smooth muscle where its effect conformed with that observed in striated muscle On the other hand it did not affect the lactic acid production of smooth muscle the behavior of which thus differed in this respect from that of striped muscle

Without addition of insulin isolated stomach muscle from rabbit in contrast to bovine mesenteric artery showed no synthesis of glycogen. Since however the possibility that the arterial preparations utilized insulin already fixed in the tissues cannot be precluded the question of whether smooth muscle has the capacity to synthesize glycogen in the absence of insulin still remains to be answered

This investigation was a ded by grants from the research foundat on of th Swed h Diabetes *Association and the Swedish Nat onal Association against Heart and Chest D seases. Th authors are ind bted to* Mr T Dzfdin and Mr N N Amos for techn cal ass stance

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From the Department of Medical Chemistry University of Helsinki Finland

Acetoin as a Metabolite of Ethanol

By

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Rece ved 25 Jun 196

Abstract

Hassinen I. *Aceto n as a metab lite f thanol* Acta physiol scand 1963 *57* 135—143 — Blood acetoin concentrations in rabbits were followed after administration of ethanol to animals receiving disulfiram and to control animals Disulfiram increased blood acetoin before and after the administration of alcohol No increase was observed in animals receiving alcohol alone Acetoin formation from acetaldehyde and pyruvate or ethanol and pyruvate was investigated *in vit* In liver homogenates and minced liver acetoin was formed from ethanol and pyruvate but not from ehtanol or pyruvate alone Disulfiram inhibited acetoin synthesis from theanol and pyruvate under aerobic conditions but not anaerobically or when acetaldehyde and pyruvate e e used as ubstrates Th role of acetoin in alcohol metabolism and disulfiramal ohol reaction is discussed

According to the present-day view the elimination of ethanol takes place in the liver through the acetaldehyde step to acetate as well as to acetoacetate and some other keto compounds The final oxidation obviously occurs extrahepatical ly (Forsander Raiha and Suomalainen 1958 1960 Forsander and Raiha 1960)

It has been demonstrated that the elimination of alcohol is accelerated by administration of insulin insulin and glucose or pyruvic acid (Widmark 1935 Clark Morrissey and Fazekas 1938 Westerfeld Stotz and Berg 1942) It has been suggested that pyruvate condenses with acetaldehyde produced by the primary oxidation of alcohol to form acetoin or that ethanol reacts with pyruvate producing acetaldehyde and lactate (Westerfeld *et al* 1942) In animal experiments however no acetoin was found in the blood before or after the administration of insulin and glucose or pyruvate although an in creased rate of dissappearance of alcohol could be verified (Greenberg 1942)

Disulfiram is known to decrease the endogenous respiration of the liver delay the elimination of alcohol and cause an accumulation of acetaldehyde in

the organism (Edwards 1949, Morimura 1955, Casier and Polet 1958, Hald, Jacobsen and Larsen 1945). But the role of acetaldehyde in disulfiram-alcohol toxicosis has subsequently been considered to be exaggerated because with enzymatic methods noticeably lower concentrations of blood acetaldehyde were found than with earlier methods (Jacobsen 1950, Wagner 1957). In contrast, the pyruvic acid values increase more compared with the increase of acetaldehyde, and it has been proposed that pyruvate plays a role in the disulfiram-alcohol reaction (Wagner 1957) or that the reaction is caused by a more profound disturbance of oxidative metabolism combined with overloading of the oxidative chain by alcohol (Ziegler and Meyer 1959).

Because disulfiram increases both precursors of acetoin synthesis and at the same time inhibits oxidative metabolism, it appears quite possible that after administration of disulfiram and alcohol acetoin would be found in the blood. This has already been suggested, but not experimentally checked (Järnefelt 1955, Rehak and Truitt 1958).

The purpose of the present investigation was to study the relation between ethanol and pyruvate metabolisms and the role of acetoin in disulfiram-alcohol reaction.

Experimental

Rabbits were used in the *in vivo* experiments. Disulfiram (Antabus Dumex) was administered through a rubber tube into the stomach in doses of 50 mg per kg body weight suspended in [illegible] ml of water for one week, after which an alcohol tolerance test was performed with 24 per cent w/v ethanol solution 1.5 g ethanol per kg body weight. Blood acetoin was determined according to Westerfeld (1945).

In vitro experiments were performed with rat and rabbit liver homogenates and minced rat liver. Homogenates were made in a Potter-Elvehjem teflon pestle homogenizer with 0.[illegible] per cent sodium chloride at [illegible] °C. The minced liver preparation was made by weighing a suitable amount of liver directly into the reaction vessel and cutting the tissue into the smallest possible pieces with sharp scissors. The size of the pieces was determined only by eye and by standardizing the mincing time.

All substrate solutions were made in water redistilled in an all-glass apparatus and were renewed daily. Ethanol solutions were made by diluting absolute ethanol purified for spectrophotometric purposes (Aa 5, Alko) from the Finnish State Alcohol Monopoly. The acetaldehyde was distilled just before each series of experiments, the fraction distilling over between [illegible] and [illegible] °C at normal atmospheric pressure being collected. Pyruvate solutions were made from crystallized sodium salt.

As incubation vessels 50 ml conical flasks were used, the stoppers of which were fitted with glass tubes for adjustment of the gas phase. Incubation was carried out with continuous shaking in a water bath at [illegible] °C. The total volume of the reaction mixture was 10 ml, 5 ml of which consisted of the tissue preparation, [illegible] ml of phosphate buffer and 2 ml of the substrates.

Acetoin was determined according to Westerfeld (1945). For the determination of pyruvic acid Rindi and Ferrari's ([illegible]) modification of the method of Friedemann and Haugen was used. Ethanol was determined according to Williams, Linn and Zak (1958) and acetaldehyde according to Burbridge, Hine and Schick (1950).

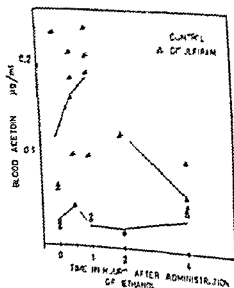


Fig 1 Effect of disulfiram tr atment nd al oh l on blood acet in
Uppe curv me ns f bl od ac toin conc ntrat n n n mals whi h had e ce ed 50 mg of disulfi am per kg body w ght f on w ek Lowe cur con trol an mals Alc hol 1 5 g per kg body we ght at time e o

Results

The normal blood acetoin concentratic n was 0 01—0 03 μg/ml The bl od acetoin values of animals receiving disulfiram were higher before the adminis tration of ethanol (0 12 μg/ml) 60 min after ethan l d sage they were about 0 19 μg/ml and returned to normal in 4 hours In animals receiving only ethanol no increase in blood acetoin concentration could be observed (Fig 1)

Tabl 1 Eff t f d ulf m adm n st d in a iso on the ynth f a et n f om a tald hyd and pyru t in at l er h m genat

Disulfir m 100 mg p d was adm n ter d at f r 7 day C p t 5 ml f l 1 m g nat 1 4 178 μmoles f a taldehyde 91 μmol f py u t Total 1 m 10 ml pH 7 4 g s pha Incubat on tim 60 min t 37 C.

N	Dis lfiram an mal	N	C nt ol n mal
	A to n μm les		A et n μm les
1	9 8	1	
	10 3	2	10.5
3	11 0	3	11.2
4	9 2	4	10 5
5	9 4		10.5

Table II Effect of disulfiram added in vitro on the synthesis of acetoin from acetaldehyde and pyruvate in rat liver homogenates

Disulfiram solution was prepared by first dissolving it in propylene glycol 1 mg/ml and diluting this with water Components 5 ml of rat liver homogenate, 178 μmoles of acetaldehyde 91 μmoles of pyruvate. Total volume 10 ml pH 7 4 gas phase air

No	Disulfiram μmoles	Acetoin μmoles	
		60 min	120 min
1	—	8 0	13 6
2	—	8 5	14 8
3	0 034	8 0	13 4
4	0 034	8 1	13.2

Table III Formation of acetoin from ethanol and pyruvate in rat liver homogenates

Components 5 ml of rat liver homogenate 1 4 344 μmoles of ethanol 1 91 μmoles of pyruvate 2 5 mg of disulfiram was homogenized in the solution at the same time as the tissue homogenate was prepared. Total volume 10 ml gas phase air pH 7 4 Incubation time 90 min at 37 C

Acetoin μmoles			
No	Controls	No	Disulfiram
1	10 8	1	6 0
2	10 7	2	5 9
3	9 8	3	8 8
4	10.[illegible]	4	8 5

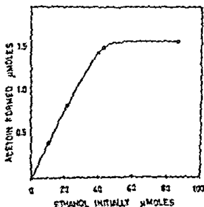


Fig 2 Effect of initial amount of ethanol on the formation of acetoin in rabbit liver homogenate 1 4 Components 5 ml of liver homogenate 1 4 91 μmoles of pyruvate ethanol as indicated Total volume 10 ml pH 7 4 Gas phase air Incubation time 90 min at 37 C.

Table IV F rmat n f a etoin f m thanol and pyruvate in minced at liver

Components 1 g of m nc d rat l e 86 µm les of ethanol 91 µmoles of pyruv te D sulfiram as indi ated was f rst diss lved in pr pyl n glycol 2 mg/ml and d lut d with wat n th cont ol vesels th same amount of diluted pr pyl ne glycol was used. Flu d volume 10 ml pH 7 4 gas phas oxygen. Incubat on tim 90 min at 37 C

No	Dis lfiram µmoles	Ac to n f rm d µmoles	Acetaldehyde formed µm les
1	—	0 20	1 1
2	—	0 18	0 9
3	—	0 24	1 0
4	0 135	0 12	0 8
5	0 135	0 15	0 5
6	0 135	0 15	0 7

Tabl V A aer bi format on of acet n f m thanol and pyruvat in m nced at l er

Compon nt 1 g of m nc d r t l ver 86 µmol s of ethanol 45,5 µmoles f pyru at Disulf ram as first dissol d n pr pyl n glycol diluted in water nd dd d a nd cated. Flu d lume 10 ml pH 7 4 gas phase nitrogen. Incubat n t m 90 at 37 C

No	Disulfiram µmoles	A et n µm les
1	—	0 48
2	—	0 56
3	—	0 50
4	0 068	0 60
5	0 068	0.54
6	0 068	0 44
7	0 068	0 46

In vitro the formation of considerable amounts of acetoin from acetaldehyde and pyruvate could be demonstrated in liver homogenates Disulfiram ad ministered *in v o* or added *in vitro* had no effect on acetoin synthesis when acetaldehyde and pyruvate were used as substrates (Table I and II)

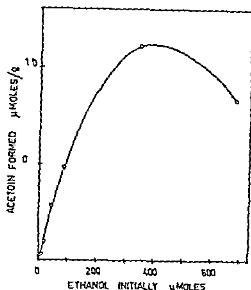


Fig 3 Effect of initial amount of ethanol on acetoin format on in minced r t l er Components 1 g of m nced rat li er 91 *μmoles of pyruvate* ethanol as indicated. Fluid volum 10 ml pH 7 4 Gas phase air Incubation time 90 min at 37 C.

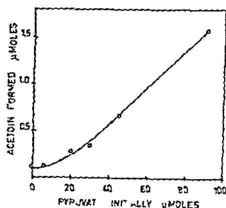


Fig 4 Effect of initial amount of pyru at on acetoin format n in minc d rat liver Components 1 g of min ed rat l er 8 moles of than l pyru at a ind cat d Flu d olume 10 ml pH 7 4 gas phase a r Incubat on tim 90 min at 37 C

When ethanol and pyruvate were used as substrates it was found that acetoin is not formed either ethanol or pyruvate alone In the p esence of pyruvate the amount of acetoin after incubation had increased to a saturation value when the concentration of ethanol was increased (Fig 2) Disulfiram in hibited the synthesis of acetoin from ethanol and pyruvate under aerobic conditions (Tabl III)

Minced liver tissue also synthesized acetoin from ethanol and pyruvate Comparison of the amount of acetoin formed with the amount formed by homogenates revealed that the former was smaller obviously because of the smaller surface area of the particles

T ble VI Effe t of oxygen on a et n synthesis from ethanol and pyruvate in m ced at liver

Compon nts 0 b g of minced rat li er 343 μm les of ethanol 91 μm les of pyruv te. Flu d v lum 10 ml pH 7 4 Incubat on time 90 min at 37 C

No.	Gas phase	Ethan l utilized μmoles	Pyruvat utilized μm les	Acet n formed μm les
1	Oxygen	13 1	56 7	0 17
2	O ygen	11.2	48 7	0 25
3	Oxygen	11 6	47 4	0 0
4	Air	10..	48 4	1 36
5	Air	9.2	43 9	1 13
6	Air	12 0	61.2	1 03
7	N trogen	8 0	38 8	1 9
8	N trogen	11.2	39 6	1 9?
9	N trogen	9 8	43 0	1 86

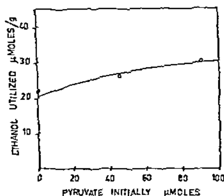


Fig 5 Eff ct f pyru t n th utilizati n f th l in min d rat l er
Components 1 g of min d t l er 86 μm les of than l pyru a as indicated Fluid olum 10 ml pH 7 4 gas phase air Incubat n tim 90 m t 37 C

Disulfiram added *in vitro* decreased the synthesis of acetoin from ethanol and pyruvate aerobically but no effect could be demonstrated under anaerobic cond tions (Table IV and V)

Minced liver did not synthesize acetoin from either ethanol or pyruvate alone When the concentration of ethanol was increased the amount of acetoin formed also increased but at concentrations over 1 6 per thousand decreased possibly because of the toxic action of ethanol (Fig 3 and 4)

In experiments with different gas phases the amounts of acetoin at the end of the incubation period were smaller when oxygen was used as the gas phase when nitrogen was used the amounts of acetoin increased (Table VI)

The amounts of acetaldehyde at the end of the incubation period were of the order of magnitude of 1 μmol

Pyruvic acid moderately increased the elimination rate of ethanol (Fig 5)

Discussion

The present investigation confirms previous observations that the elimination of ethanol is accelerated by pyruvic acid That acetoin has not been found in blood during alcohol detoxication does not rule out the possibility that acetoin is a metabolite of ethanol The amount of an individual metaboblite in a biochemical reaction chain at a steady state dynamic equilibrium depends on the kinetics of all the different reactions affecting that metabolite

The increased formation of acetoin when the partial pressure of oxygen is decreased may depend on a decrease in the oxidative elimination of acetoin The anaerobic coupled oxidation reduction between ethanol and pyruvic acid proposed by WESTERFELD (1943) supplies the acetaldehyde necessary for the synthesis of acetoin even under anaerobic conditions

Acetoin synthesis is considered to be a nonoxidative decarboxylation di phosphothiamine and Mg^{++} ions functioning as co factors A complex of thiamine pyrophosphate and pyruvic acid is formed active acetaldehyde which reacts with pyruvic acid or acetaldehyde to form acetoin The animal organism is not able to synthesize acetoin from pyruvate alone (REED 1953 JARNEFELT 1955 HOLZER and BEAUCAMP 1961)

It has been suggested that the mechanism underlying the effect of disulfiram is inhibition of thiamine pyrophosphate (WAGNER 1957) Since disulfiram whether added *in vitro* or administered *in vivo* does not affect the synthesis of acetoin either from acetaldehyde and pyruvate or from ethanol and pyruvate anaerobically there cannot be any question of universal inhibition of thiamine pyrophosphate Because the effect is observed only under aerobic conditions the effect must be exerted on the first step of alcohol oxidation

The results do not exclude the possibility of inhibition by disulfiram of the oxidative decarboxylation of α keto acids but the effect cannot be due to inhibition of thiamine pyrophosphate as such but rather inhibition of the oxidative part of the reaction

The increased values of blood acetoin after administration of disulfiram and alcohol are obviously caused by high pyruvate concentrations in the blood

Acetoin probably has nothing to do with the disulfiram alcohol reaction The toxicity of acetoin is only 25 per cent higher that that of ethanol and additive with it (GREENBERG 1943) The observed concentrations of acetoin are so low that they hardly can cause symptoms

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From the Institute of Neurophysiology, University of Copenhagen, Denmark

Birefringence of Isolated Muscle Fibres in Twitch and Tetanus

By

ARTHUR EBERSTEIN[1] and ANNELISE ROSENFALCK

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Abstract

EBERSTEIN, A. and A. ROSENFALCK. *Birefringence of isolated muscle fibres in twitch and tetanus.* Acta physiol. scand. 1963. 57. 144—166. — The photoelectric measurement of changes in birefringence in single frog muscle fibres during isometric contraction is described. Birefringence, mechanical tension and intracellular action potential were recorded simultaneously. During a twitch birefringence decreased before tension was developed and reached minimum before tension reached maximum. The decrease in birefringence was 9 per cent at equilibrium length; at greater lengths the change in birefringence was less, as was twitch tension, but even when the sarcomeres were stretched to 3.6 and 3.8 μ a decrease in birefringence occurred. Double peak changes in phase difference, which have been described in whole muscle during isometric twitches, were not observed unless the fibre twisted in the light path. The decrease in birefringence was the same during twitch and tetanus. The decrease in birefringence during contraction may be due to an increase in the number of cross-links between minute structural elements, or to a displacement of water within the fibre.

The initial phase of contraction has been studied by measuring longitudinal stiffness (GASSER and HILL 1924, BUCHTHAL and KAISER 1944), torsional stiffness (STEN-KNUDSEN 1953), latency relaxation (A. SANDOW 1944), heat production (A. V. HILL 1949, 1953 a), light diffraction (D. K. HILL 1953) and volume (ERNST 1925, MEYERHOF und HARTMANN 1934 and ABBOTT and BASKIN 1962). These changes were all found to begin before the development of positive tension.

[1] Postdoctoral Fellow of the National Science Foundation 1957—58. Postdoctoral Fellow of the United States Public Health Service 1958—59.

Muscle acts as a birefringent medium when light passes the orderly arranged long chain molecules myofilaments and myofibrils A recording of changes in birefringence at a high time resolution may thus add to our knowledge of minute structural changes during activity A decrease in birefringence of whole muscle during isometric twitch has been described (VON MURALT 1932 BOZLER and COTTRELL 1937) Von Muralt showed that birefringence reached minimum before tension reached maximum but his method did not allow the onset of the two events to be compared

The detailed time course of the change in birefringence of small fibre bundles was measured photoelectrically in preliminary experiments (ROSENFALCK and ROSENFALCK 1957) To ensure that the change in phase difference was not caused by mutual displacement of the fibres the experiments presented in this report were performed on single fibres Moreover the relation between changes in birefringence and light intensity is simple when the preparation is thinner than 168 μ (see p 5) Fibres were selected to have a constant diameter and phase retardation along the major part of their length It was the aim of this study to correlate the time course and magnitude of birefringence and force at different initial lengths during isometric twitch and tetanus In a number of experiments the intracellular action potential was recorded as well

Method

THEORY

M as r me t of l an e   bi efri g nc by me s eme t of light intensity

To measure th changes n b fringence during contract on a s ngl muscl fibr as uspend d n an opt l slit illuminated by a parall l bundl of linea ly polar zed monoch omat l ght (I_p n F g 1) Entering the fibre the compon nt of th ele tri v ct pa llel to th fib axis (efract ve ndex) is t ansmitted mo slo ly than th compon nt perp nd ular to th fibre ax (ef ctiv ndex n_o) After passa of th fib e the e s a phase d ff rence φ betw een thes el ctri fi ld v tors An analyze pl ced belo the f b ngles out th l ght ompon nt n on d ection and the components of the le tric field ct rs n th d re tion interfere su h th t the light ntens ty aft passag of th analyz is a fun tion of the phas diff n φ

Assum g a re tangul c ss t on of the f b (th ckness b dth 2a Fig 1 A) the ntens ty pe un t fib e l ngth is

$$I = 2 \ I \left\{\cos \beta - \sin 2\gamma \ \sin 2(\gamma - \beta) \sin \frac{\varphi}{2}\right\} \quad (1)$$

wh re $\varphi = \frac{2\pi}{\lambda}(\ - \ ) 2b$ the phase diff ren e $n_e - n$ the b refringence of th f b and λ the el ngth of th l ght I is p opo tional to th intens ty of the light w th which th f b e is lluminat d and depend on the diffraction scattering and ab orpt n of th f b and th bsorpt on of the polari e and an lyze (Pol ro ds) I the l ght t ns ty wh ch would pass th analyzer pe un t area if a fib vith ut b efri g n re pl ced bet en Polaro ds with th r planes of pol r zat on pa all l to e ch other (parallel Pola o d)

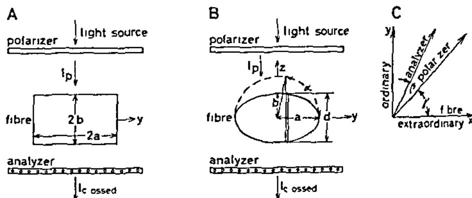


Fig 1 *Light path through the muscle fibre*

The muscle was illuminated by monochromatic polarized light with an intensity I_p. After passage of the fibre the light intensity was $I_{crossed}$ (β 90)

A Assumed rectangular cross section of the muscle fibre (thickness 2b width 2a)

B Assumed elliptical cross section of the muscle fibre (largest thickness 2b largest width 2a) d is the length of the light path in the narrow fibre segment given by d = 2b ... z axis is opposite to the light direction y axis is perpendicular to the direction of the light

C The fibre was placed with its longitudinal axis in the direction x at an angle γ to the plane of polarization of the polarizer The angle between the plane of polarization of the polarizer and analyzer was β

The angle γ between the fibre and the plane of polarization of the polarizer (Fig 1C) was chosen to give equal light components parallel and perpendicular to the fibre axis $\left(\frac{\pi}{4}\right)$ thus provided diffraction scattering and absorption are the same for the ordinary and extraordinary beam the light was circularly polarized after passage through the fibre The angle β (Fig 1 C) between the planes of polarization of the polarizer and analyzer was either $\frac{\pi}{2}$ (crossed Polaroids) or zero (parallel Polaroids) The intensity of light per unit fibre length for crossed Polaroids is derived from equation (1)

$$I_{crossed} = 2aI \sin \frac{\varphi}{2} = aI \left(1 - \cos\frac{2\pi}{\lambda}(n - n)2b\right) \tag{2}$$

for parallel Polaroids

$$I_{parallel} = 2aI \cos \frac{\varphi}{2} = aI \left(1 + \cos\frac{2\pi}{\lambda}(n - n)2b\right) \tag{3}$$

Therefore if the thickness 2b of the fibre remains unaltered during the isometric contraction birefringence ($n - n$) can be measured photoelectrically For crossed and parallel Polaroids the changes in light intensity should then be equal in magnitude and opposite in direction

In the following it is taken into account that the cross section of the muscle fibre is nearly elliptical[1] With crossed Polaroids the intensity of the light transmitted by a narrow segment (S) of the muscle fibre is (Fig 1 B)

$$dI_y - aI \left(1 - \cos\frac{2\pi}{\lambda}(n - n) d\right) \tag{4}$$

These calculations were made by A F Huxley who kindly placed his notes at our disposal.

where d is the thickness of the fibre segment The intensity of the light which leaves the analyzer per unit fibre length is therefore

$$I_{crossed} = \int_{-a} dI_y = aI \left(1 - \int \frac{\cos\frac{2\pi}{\lambda}(n - n_o)d}{a} dy \right) \tag{5}$$

a is the fibre diameter perpend cular to the light path
Substituting in (5)

$d = ?b \sin\alpha$ and $y = \quad \cos\alpha$ (6)

wh re 2b is the fibre d ameter parallel to the light path

$$I_{crossed} = aI_o\left(1 + \int_{\frac{\pi}{2}} \cos\left(\frac{2\pi(\quad - n) ?b}{\lambda} \sin\alpha\right) s\ n\alpha\ d\alpha\right)$$

$$= aI\left(1 + \int_{\frac{\pi}{2}} \cos(\Phi \quad \sin\alpha)\ s\ n\alpha\ d\alpha\right) \tag{7}$$

where $\Phi = \frac{4\pi b(n - n\ )}{\lambda}$ is th phase difference at th th kest point of the fibre

Integrating (7) by parts one obtains

$$I_{cr\ ssed} = aI\ \Phi \int^{\frac{\pi}{2}} \cos\alpha \sin(\Phi\ \ s\ n\alpha)\ d\alpha \tag{8}$$

Introdu ng $\beta = \frac{\pi}{2} - \alpha$ we get

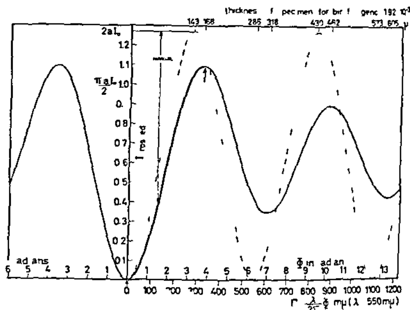
$$I_{crossed} = \frac{aI_o\pi}{2} S\ \ (\Phi\ ) \tag{9}$$

$$\text{here } S\ \ (\Phi\ ) = \frac{2\Phi}{\pi}\int^{\frac{\pi}{2}} \text{in}(\Phi\ \ \cos\beta)\ s\ n^2\beta\ d\beta \tag{10}$$

is the Struv funct on of f rst orde (JAHNKE and EMDE 194 p ?19)
The sum of $I_{crossed}$ and $I_{parall\ l}$ is $2aI$ (equations (?) and (3)) Hence

$$I_{parall\ l} = \frac{I_o\pi}{?}\left(\frac{4}{\pi} - S\ \ (\Phi\ )\right) \tag{11}$$

The relat n b tw een l ght ntens ty and phase difference for crossed Polaro ds for f bres with elliptical and rect ngula cross s ctions is gi en in F g ? A gi en value of light intens ty corresponds to ev ral post e and gati e values of phase d fference W th a b ef ngence at rest of 1 9? × 10⁻³ (se p 14) and λ = 5?0 mμ the peak of the curv at Φ — 3 7 rad ans would correspond to a f b e th ckness of 168 μ W th few except n th single f bres had a d am te of less than 168 μ Henc a decrease in I



F g 2 L ght intens ty after pas age f cross d Pola oids and a bi efr ngent p c men as a f nctı of phase differenc calculated f an ell ptic 1 (full line) and a rectangular cross sect on (dott d lin)

Abscissae
(bel w) phase differen Φ of th th kest part of th fibre in r d an and correspo ding phase r tardation Γ

$$\left(\Gamma = \frac{\lambda}{2\pi}\Phi \text{ m}\mu \text{ fo } \lambda = 550 \text{ m}\mu\right)$$

above) thickness of th specim n assuming the bir f ing nce to be 1 9 × 10^{-3}

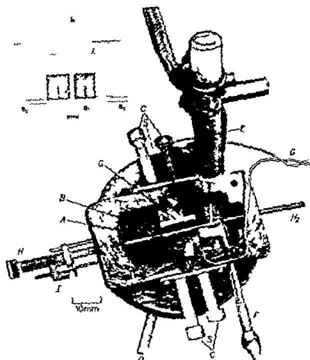
intens ty ıth crossed Polaroid corresponds to a decrease in phase difference S n e the decrease in light intens ty had a smooth course throughout contraction and did not exceed 15 per cent (see p 17) changes n phase difference vere confined to the port on of the curve (Fig 2) wh ch is indicated by the arrows (0—3 7 rad ans ell ptical f bre)

EXPERIMENTAL ARRANGEMENT

1) *Experim ntal hamber* (Fig 3 the single muscle fibre (A) was mounted in Ri ger s solution betv een two plates (B) nade of black glass 13 mm long and 2 mm high hich could be mo ed close and parallel to the fibre by four screws (C) T o open gs in the floor of the hamber were co red by thin (0 2 mm) glass plates 4 mm square and 4 mm apart o that two eparate sections of the muscle f bre could be examined

2) *The Ringer s solut o* ontained 115 mM NaCl 2 7 mM KCl 1 1 mM glucose 1 8 mM $CaCl_2$, 4 2 mM NaHCO and 3 per cent Dextran which provided a colloid osmot c pressure of 86 mm H O The olution wa bubbled with 1 per cent CO_2 and 99 per cent O_2 and the pH was between 7 3 and 7 6 Tubocurarinechloride (20 μg per ml) was added to the Ringer

3) *The temperature* of the Ringer s solut on was maintained at 7 C or at 17 C by means of a thermistor which co troll d the circulation of the glycol water mixture around the chamber (D)



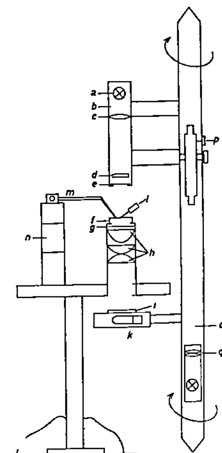
F g 3 E pe ment l h mbe Inset oss s t on n th pl n S—S

Th is l t d muscl f b (A) was pl d n Ringer solut n (inset (A)) n a l t l m ted by plates (B) dju tabl by crew (C) to duce byp ss ng l ght (ns t B_1 cons ted f black glass B f bla k plas) On t nd n d f th f b was onn ted to a f transd c RCA 5 34 (E) and th th r th adjust l l micr m t crew (I) H nd H s rved to untwist the fib bef th perim n H w l drawn bef th co d ng f l

Th fib wa th t m l t d tr n rs ly f m h l t odes (G) t on nd f m the l t odes (F)

Cool g l qu d was passed th gh th d bl wall of th h mber f m th nl t (D)

4 *Opt l arran ement* (F g 4) th light sou c (a) as an a cooled tungsten lamp (1? Volt Os am 55 5004) nn t l to an 18 Volt DC sou ce A parall l bundle of l ght f om d oll mat ng l n (pass d th ough a 550 mμ nterf rence filter (d) a polarizer (e) and th mu l f bre in th exper mental hambe (f) To avo d lens eff cts at the urfa f th flu d urround ng th f b e th spac of th experimental chamb r above th sl t (h n F g 3 t) wa f ll d w th Ring r solut on The polarizer F g 4 (e) and analyz (g e plac d th th planes of polarizat on at an angle of 45 to the fibr H nc th pola iz had t plane of pol rizat on either parallel or perpendicular to that of the nalyze A t of oll ating l nses (h) placed 10 mm belo the fibre coll ct d th d ff a ti n sp t a up to the fourth order and formed an image of the fib e on a pl t of m lk glass (n front f the multiplie phototube (k) (Mazda 27 M3) The m lk glas as d ns nough to l m nate th eff ct of the unequal sens t vity across th cath od of th multipl e phototube To measu the onset of the change in l ght ntens ty th s gnal to-no e ratio was inc ased by replacing the milk glass with a ground glass plat Lat nc es of larg gnals m asu ed suc ess vely w th a m lk glass and with a ground gla plat were d ntical



F g 4 S t up for the re rding of the light intens ty change force and ntracellular act on potent al

The l ght source was a tungsten l mp (a) Th l ght pa ed th chopper (b) th c ndenso lens (c) th interfer n e filter (550 m/) (d) the pol zer (e) the muscle fib e in the experimental hamber (f) and the an lyzer (g) After pass ge of th analy er th l ght was collect d by lenses (h) on a milk glass ( ) in f ont f mult pl er phototube (k Ma da 27 M3) One tendon end f th fib e was connected to the f rce transducer (l RCA 5734) for the recording of te on For ec d g of the act on p tential the microelect ode (m) was nserted nto th fibr by means of th m rom nipul tor (n)

Fo ob rvation the l ght o ce (a) was r placed by a m rosc p (p) by t n ng the stand (o) the l g t s u c (q) was used f r ob rva t on photom rography nd for measu em t of th maximum phas r t rdat on of the rest ing fibre (B bin t compens t r)

5) *Record ng of the changes in li ht intensity* The multiplier phototube was supplied by 500 Volts stabilized voltage Dynodes 8 and 9 could be shortcircuited to reduce the photocurrent when very large signals were obta ned The output of the phototube was amplified and displayed on one of the two beams of a cathode ray oscilloscope To avoid resetting the zero level of a DC amplifier for each intensity level an AC amplifier was used with a time constant (T) of 1 5 sec and in the preliminary experiments of 0 3 sec In addition to the recorded l ght intensity changes (I) a corrected time course (I) is given in the figures Corrections were made according to $I = I + \frac{1}{T}\int I_t dt$ Also the upper frequency limit was adjustable An upper l miting frequency of 900 cps d d not distort significantly the shape of the s gnals but reduced the noise level so that a 0 5 per cent change in light intensity could be discr minated The noise originated mainly from the light source the dark current of the multiplier tube and the noise of the amplifier were negligible

6) *Record n of isometric tension* One tendon end of the fibre was attached to the elongated pin of an electromechan cal transducer (RCA 5734) (Fig 3 E) the output of wh ch was ampl fied and displayed on the other beam of the cathode ray oscilloscope Chan es in tension greater than 0 7 mg (0 00? P) could be discrim nated

7 *The action potent al* was led off intracellularly with a conventional glass microelectrode filled with 3M KCl connected to a DC amplifier via a cathode follower The action potential the change in light intensity and the tens on developed were recorded s mul taneously by splitting one beam of the oscilloscope with an electronic switch

8) *The st mulus* consisted of a single rectangular current pulse with a duration of 1 msec or of a train of pulses (20—40 per sec) The stimulus was appl ed either trans ver ely over the entire length of the fibre or to one end of the fibre The transverse field was produced by applying the curr nt pulses through t o sheets of platinum 14 mm long and 2 mm wide one placed below and the other above the plates wh ch formed the slit (Fig 3 G) Two platinum electrodes (F g 3 F) 1 mm long 110 μ thick and 2 mm apart were used to stimulate one end of the fibre

With transverse stimulat on the stimulus was gradually increased unt ll the tension developed was maximum a st mulus strength of 10 per cent above this value was used in the experiments

9) *Measu ement of fib d mensions and phas ta dation* The fib e diameters were deter mined f om enlarged micrographs fo the determinat on of the diameter pa allel to the l ght b am the fib e was turned 90 The degree of stretch was determ ned by measuring the sa comere length from enlarg d m crographs The phase retardat on (Γ) of the thickest point of the rest ng f bre was measured by means of a Babinet compensator For these measu ements and to insert the m croelectrode nto the fibre a microscope was rotated into position over the fibre (F g 4 p)

PROCEDURE

1) *P par t n and mo nt ng f th f b* S ngle fib es we e isolated from the semitendinosus muscle of *Rana temporaria* o *Rana e culenta* The muscle was mounted by its tendons in a perspex dish conta n ng R nger olution at 16 C The muscle could be rotated about ts long tud nal axis so that the fibres could be more easily separated and inspected for injury unde high magnif at on A s ngle fibre was eparated f om the v hole muscl v th f ne tissors and for eps and as cleaned of excess tissue along ts ent re length The isolated fibr wa lifted b th tendons and transf rred from the preparation d sh to the chambe (Fig 3 An alumin um cyl nder (6 mm long 0 7 mm d ameter) was pressed onto ea h t nd n nd of th fibr one cylinder wa fix d to a platinum od (H_1) and the other sl pped through an alum n um tube attach d to the transducer arm (E) and th n f x d by queez ng th open nd Twist ng of the f bre during contraction was p e nt d bv rotat ng th rods H and H

2) *P ord g b rvat* After pla ing the chamb betw een the two Polaro d plates (F g 4 g) th natural l ngth of the fibre was adjusted bv means of a m crom ter cre (F g 3 I) untill the l b as just taut th a load of about 1 mg (0 003 P) It was ess nt al to p ev nt th f bre f m twisting ev n l ghtly during contraction To r duc l ght pass ng bes d th fib the sl t was narrowed to with n 50 μ on each ide of it Tl abs nce of art facts du to f b e movement to changes in diff act on absorption and catt ring du ng ont a t on was then ascertained by

a *cord ng changes n l ght nt nsity* equal n amplitude and oppos te n sign w th cros ed and pa all l Polaro d

b) as erta n ng that the e v as no hang n l ght ntens ty hen the f bre was llum nated with non polarized l ght (ee p 14)

3) *Re d ng and measur ment* Fo latency measurements ten twitch contractions were recorded at 5 sec interval To determine rise t me and magn tude of the changes in b ref ng n and t ns on f ve cord ngs wer taken two with the Polaro ds crossed two w th th Polaro ds parall l and one with th pol rizer r moved The reco d ng p ocedure was foll wed both for trans erse and end st mulation and with th fibre at d fferent

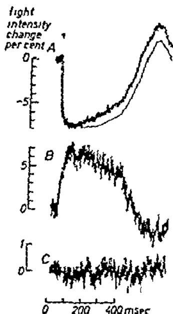


Fig 5 To show that the *changes in light intensity* associated with a decrease in birefringence during a twitch were identical in shape and of opposite sign with Polaroids crossed (A) and with Polaroids parallel (B)

The phase retardation at rest was 190 mμ and the change in light intensity corresponded to 5 per cent decrease in birefringence *The thin line gives the time course of* (A) corrected for the distortion by the *time* constant of the amplifier (1 5 sec)

C Non polarized light passing by and through the fibre during a twitch remained unaltered indicating that the change in light intensity caused by the decrease in birefringence was not distorted *by a displacement of the fibre* or by changes in light scattering and absorption (Sarcomere length 2 4 μ 7 C transverse stimulation)

lengths At each length the phase retardation and the sarcomere length were determined for the resting fibre With the fibre between crossed *Polaroids* the light intensity changes were expressed in per cent of the light intensity passed by the fibre at rest *it* was measured by chopping the light mechanically (Fig 4 b) at a frequency of 110 per sec The corresponding change in phase retardation was *determined from the theoretical curve* for the elliptical fibre (Fig 2) The changes in light intensity are equal in amplitude but opposite in sign when the *fibre is* between crossed and parallel Polaroids Therefore the change in light intensity with the fibre between *parallel* Polaroids was also expressed in per *cent* of the light transmitted through the resting fibre between crossed Polaroids The change in light intensity which may be caused by scattering and diffraction as well as changes in light passing beside the fibre were expressed in per cent of the intensity of non polarized *light* transmitted by the fibre at rest

Due to the light which bypasses the muscle fibre in the slit the intensity ($I_{non\text{-}polarized}$) of non polarized *light transmitted* by the resting fibre itself could not be measured directly nor could the light intensity be *measured* with parallel Polaroids ($I_{parallel}$) without *interference* of bypassing light

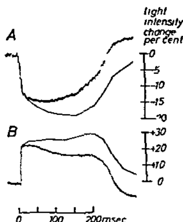
In five experiments bypassing light was eliminated by narrowing the slit and $I_{non\text{-}polarized}$ and $I_{parallel}$ could be measured

It was found that

$$I_{non\text{-}polarized} = 1.4 \, (I_{crossed} + I_{parallel}) \qquad (12)$$

That $I_{non\text{-}polarized}$ was not simply the sum of $I_{crossed}$ and $I_{parallel}$ was due to the absorption of light in the polarizer Since bypassing light usually could not be avoided $I_{parallel}$ was determined from the value of $I_{crossed}$ and the phase retardation at rest (Fig 2) $I_{non\text{-}polarized}$ was then calculated from equation (12)

The fraction of the light transmitted by a 100 μ fibre illuminated by non polarized light is approximately 50 per cent (BUCHTHAL, KNAPPEIS and SJOSTRAND 1939) Hence the intensity of the light which would be transmitted through a slit of the same width



F g 6 Change in l ght intens ty fo crossed P laro ds companying a decrease n b ref gen e during an isom tr twitch when th initial phas retardat n of th fib was l w (A) nd high (B)
T btain a low initial ph se eta d t n (23 mμ) the llipt cal fib was pl d with th minor axis (125 μ) p ll l t th light p th t obtain a h gh ini tial phase etard t n (460 mμ) the fib was placed with th maj a is (238 μ) p allel to th l ght p th
Th irr gula h p and th gre t m gnitud of the l ght int n ty hang indicat th t th fib twisted l ghtly during ntr ct on Th xperim nt was ther f e n t includ d n th m in m t al (T ble II and F g 11) Th thin lines gi th t m cours orr t d f th distortι n by th tim o istant of th ampl fi (0 3 se) (Sarco n length 2 1 μ 12 C t ansverse stimulat n)

as the fibr in the ab nce of the fib e as 2 I polarized Changes in light ntens ty n n npolari ed light due to scattering diff action and absorpt on were expressed n pe c nt of I p l rized
Changes in bypass ng light w hi h w ere added to the aforementioned changes w ere also exp essed n per cent of I on polarized although they should have b en expressed in per cent of 2 × I no p larized This gives a saf ty factor n the estimat on of the artefact

Results

EVIDENCE THAT THE CHANGE IN LIGHT INTENSITY IS A MEASURE OF THE CHANGE IN BIREFRINGENCE

1) As expected from theory (see p 5) the decrease in birefringence for a fibre thinner than 168 μ was accompanied by a decrease in light intensity for crossed Polaroids (Fig 5) The change in light intensity was equal in size and opposite in direction with crossed and parallel Polaroid (equations (9) and (11)) That the noise level was greater with parallel Polaroids and in non polarized light than with crossed Polaroids was due to the light which by passed the fibre in the slit

2) It could be expected from theory that the direction of the change in light intensity depended on the phase difference at rest (see p 3 Fig 2) This was shown experimentally in fibres whose major axis was greater than 168 μ and differed substantially from the minor axis When the light passed through the minor axis (phase difference at rest smaller than 3 7 radians) a decrease in birefringence was accompanied by a decrease in light intensity during con traction (Fig 6 A) When the fibre was rotated and the light passed through the major axis the phase difference was larger than 3 7 radians and the con traction was accompanied by an increase in l ght intensity (Fig 6 B)

3) Finally fibres with a phase difference at rest of about 3 7 radians showed no changes in light intensity during contraction although they developed normal tension

Table I Calculated changes in light intensity caused by twisting of the fibre

Fibre diameters in μ min.	Fibre diameters in μ max	Twisting angle degrees	Per cent change in light intensity: Non polarized	Polaroids crossed: For twist alone	Polaroids crossed: For 10% decrease in birefr	Polaroids crossed: For twist plus 10% decrease in birefr	Polaroids parallel[1]: For twist alone	Polaroids parallel[1]: For 10% decrease in birefr	Polaroids parallel[1]: For twist plus 10% decrease in birefr
A. Minor diameter of the resting fibre parallel to the light path									
120	200	0	0	0	−10.5	−10.5	0	+10.5	+10.5
		10	+ 1	+ 1.8	−10.5	− 8.5	− 1.8	+10.5	+ 9.5
		25	+ 5	− 0.4	−10.5	− 9.7	+ 0.4	+10.5	+14.7
		32	+ 10	− 1.1	−10.5	− 8.5	+ 1.1	+10.5	+18.5
		47	+ 20	− 6.8	−10.5	−11.3	+ 6.8	+10.5	+31
		90	+ 40	−39	−10.5	−3[illegible]	+39	+10.5	+72
100	120	0	0	0	−12.0	−12.0	0	+12.0	+12.0
		20	+ 1	− 0.6	−12.0	−13.5	+ 0.6	+12.0	+14.5
		36	+ 5	+ 2.2	−12.0	− 9.9	− 2.2	+12.0	+14.9
		51	+ 10	+ 0.7	−12.0	−11.3	− 0.7	+12.0	+21
		90	+ 17	+ 2.1	−12.0	− 8.4	− 2.1	+12.0	+25
B. Minor diameter of the resting fibre at an angle to the light path									
120	200	0	0	0	−10.5	−10.5	0	+10.5	+10.5
		10	− 1	− 1.8	−10.1	−12.1	+ 1.8	+10.1	+11.1
		25	− 5	+ 0.4	− 9.4	−10.4	− 0.4	+ 9.4	+ 5.4
		32	− 10	+ 1.1	− 8.1	− 9.5	− 1.1	+ 8.1	− 0.5
		47	− 20	+ 7.2	− 4.8	− 4.0	− 7.2	+ 4.8	−16.0
		90	− 40	+64	+11.1	+47	−64	−11.1	−87
100	120	0	0	0	−12.0	−12.0	0	+12.0	+12.0
		20	− 1	+ 0.6	−13.0	−11.5	− 0.6	+13.0	+10.5
		36	5	− 2.2	−11.9	−13.9	+ 2.2	+11.9	+ 8.9
		51	− 10	− 0.7	−11.9	−12.6	+ 0.7	+11.9	+ 2.6
		90	17	− 2.1	−10.2	−13.8	+ 2.1	+10.2	− 3.2

In per cent of non polarized light at rest (see p 9)
In per cent of the light transmitted by the resting fibre with Polaroids crossed.
The fibre is assumed to twist during contraction to a position where the minor diameter is parallel to the light path.

SOURCES OF ERROR

Changes in light intensity other than those indicating the change in birefringence associated with the contractile process may arise from (1) a change in light path due to twisting of the fibre (2) a change in the amount of birefringent substance due to stretch or shortening of the fibre and (3) either alone or combined with (1) and (2) a change in light scattering diffraction and absorption

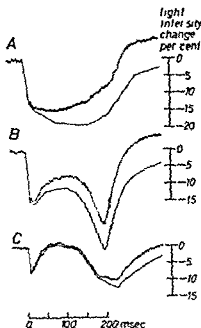


Fig. 7. To show that twisting during contraction is accompanied by a double peak change in phase retardation (crossed Polaroids).
A Isometric twitch of a slightly twisted fibre.
B and C Additional twisting during contraction caused by rotation of one end in the resting fibre.
Minor diameter of the fibre 125 μ (parallel to the light path), major diameter 460 μ, the phase retardation at rest was 235 mμ. (Sarcomere length 2.1 μ.) C transverse stimulation.
The thin lines give the time course corrected for the distortion by the time constant of the amplifier (0.3 sec).

1) *Twisting of the fibre* (Table I). Since most fibres have an elliptical cross section a twisting during contraction causes not only a change in light path through the fibre and therefore a change in phase retardation but also a change in the amount of light not traversing the fibre. The light intensities to be expected in a fibre placed with its minor axis at different angles to the light path were calculated by numerical integration of the contributions from small segments of the fibre at rest and during contraction (equation (4)). With crossed Polaroids and an angle of twist of less than 50° the change in light intensity due to twisting during contraction may alter the decrease in birefringence by at most 2 per cent. With an angle of twist of 90 degrees and a highly elongated cross section the change in light intensity is predominantly due to twisting. With parallel Polaroids light passing beside the fibre compensates for or adds to the change in light intensity caused by the change in light path and the change in light intensity due to twisting is insignificant as long as the angle of twist is less than 30°. In fact if one end of a fibre with a highly elongated cross section was twisted 30° the light intensity changed in a more complicated way during contraction than predicted from calculation (Table I, Fig. 7). This was probably due to the variation in phase retardation along a twisted fibre.

To make certain that twisting during contraction did not obscure the changes in light intensity due to changes in birefringence fibres were discarded in which the change in light intensity in non polarized light exceeded 1 per cent of the

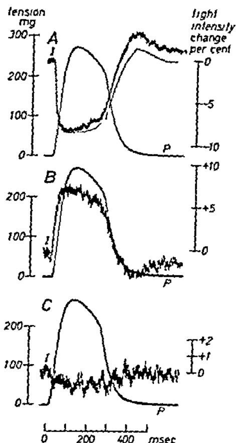


Fig 8 To show the change in light intensity (I) and tension (P) during an isometric twitch. (A) crossed Polaroids (B) parallel Polaroids, (C) non polarized light The phase retardation at rest was 26 mμ and the change in light intensity corresponded to an 8 3 per cent decrease in birefringence (Sarcomere length 2 4 μ C transverse stimulation
The time constant T of the amplifier for recording of light intensity changes was 1 5 sec The thin lines gives the time course corrected for the distortion by T

light transmitted by the fibre at rest (see p 9) The absence of twisting is furthermore indicated by the numerically identical changes in light intensity during contraction with crossed and parallel Polaroids

In most instances it was possible through microscopic observation to adjust the fibre so that there was almost no twisting

2) *Shortening of the isometrically contracting fibre* The maximum shortening during an isometric contraction which could occur due to movement of the lever of the transducer was 0 5 per cent of the fibre length The corresponding 0 25 per cent increase in fibre diameter caused a 0 5 per cent increase in light intensity with crossed Polaroids and an insignificant decrease with parallel Polaroids In non polarized light the increase in thickness due to shortening was associated with a decrease in light passing beside the fibre of at most 0 25 per cent

3) *Diffraction scattering and absorption* Light intensity changes due to diffraction scattering and absorption are easily detectable because they have the same direction with crossed and parallel Polaroids and in non polarized light The

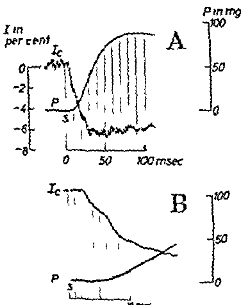


Fig 9 To h w that th d creas n b fringenc du ng a twitch begins e l er a d at a h gher rat than the ncr as in isom t n t n n

B was taken with a 4 t mes exp nded tim base Th I w n in B th n in A was obta n d by epl c ng th milk glass th I ss d as ground glass (p 6) (Phase ta dat n at est 195 m/ maj diam ter 100 μ sa comere l ngth 2 5 μ 7 C t ans rse si m lat)

Th t m onst t f th mpl f f r ec d ng of l ght int n ty hanges was 7 5 s Th thin line in A gi es the tim ourse orr t d for th distort n by T Th was n di tors n by th t m on tant n B

change in absorption during contraction is only of the order of 0 1 per cent and therefore negligible as a source of error (BUCHTHAL et al 1939) More important are changes in diffraction or scattering and the optical arrangement was designed to reduce these sources of error (see p 6)

In summary the following criteria ascertained that a change in light intensity during contraction corresponded to a change in birefringence

1 The change in light intensity with crossed and parallel Polaroids was equal in size and opposite in direction

2 The change in light intensity with the fibre in non polarized light was less than 1 per cent

BIREFRINGENCE AND THICKNESS OF THE RESTING FIBRE

The birefringence of the resting fibre averaged $1\ 92 \pm 0\ 03 \times 10^{-}$ (17 fibres)

The minor diameter of the 61 fibres examined was 50 to 160 μ in 41 fibres 50 to 120 μ the major diameter was 80 to 200 μ in 48 fibres 80 to 180 μ in 5 fibres above 200 The ratio between the major and the minor diameter ranged from 1 to 2

BIREFRINGENCE OF THE CONTRACTING FIBRE

1) *Chang i n bi ef in enc and tens on r c ded simultaneously during an isometric twitch* A typical record of the changes in light intensity and tension during a twitch is shown in Fig 8 The change in light intensity had equal size and on-

Table II Time relationship between the change in birefringence and the twitch tension

Latencies, time to half peak and to peak amplitude

	Transverse stimulation				End stimulation			
	7 C		12 C		7 C		12 C	
	t_1	τ_1	t_1	τ_1	t_1	τ_1	t_1	τ_1
S E. M	8 4 ± 0 4	11.5 ± 0.6	5 7 ± 0 4	6.8 ± 0 3	10.6 ± 0.5	15 3 ± 0 7	8.2 ± 0.5	11 4 ± 0 7
$t_1 - \tau_1$	3 2 ± 0.5		1 1 ± 0.2		4 7 ± 0 7		3.3 ± 0.5	
No of experiments	14		10		8		9	
	t_2	τ_2	t_2	τ_2	t_2	τ_2	t_2	τ_2
S E. M.	19 1 ± 1 4	32 6 ± 2.3	17 7 ± 1.2	23 7 ± 1.3	23 6 ± 1 9	35 0 ± 3 1	18 6 ± 0.8	26 0 ± 1.5
No of experiments	10		9		5		7	
	t_3	τ_3	t_3	τ_3	t_3	τ_3	t_3	τ_3
S E. M.	49 1 ± 6.2	96.0 ± 6.8	29 7 ± 3 5	60 8 ± 4 3	64.2 ± 4 [illegible]	9[illegible] 4 ± 11 0	47 6 ± 2 9	64 3 ± 4 6
No of experiments	10		9		5		7	
Sarcomere length	2 44		2 4[illegible]		2 50		2.53	

t_1 Latency of decrease in biref ngence.
τ_1 Late cy of the pos ti e tens on.
t_2 Time f om th stimulu to half the maximum decrease n birefringence
τ_2 Tim from th stimulus to half th peak tens on.
t_3 Tm from the stimulus t the maximum d crease in b refringence.
τ_3 Time from th stimulus to th peak tension.

posite direction with the Polaroids crossed and parallel and there was no change in light intensity in non polarized light Except for the faster initial rise and for a slight increase in phase retardation at the end of the twitch the time courses of the decrease in phase retardation and of the increase in tension were similar

Latency and rate of rise With transverse stimulation at 7 C the first change in birefringence occurred 8 4 ± 0 4 msec after the stimulus the onset of tension development was 3 2 ± 0 5 msec later (sarcomere length 2 44 ± 0 04 μ) When one fibre end was stimulated so that the activity was conducted to the region in which birefringence was measured birefringence started to decrease 10 6 ± 0.5 msec after the stimulus and tension started to develop 4 7 ± 0 7 msecs later (7 C sarcomere length 2 4 — 2.5 μ Table II)

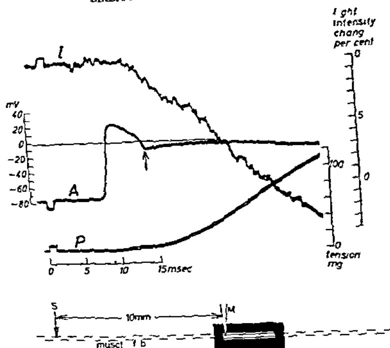


Fig 10 The time relation between the intracellularly recorded action potential (A) the onset of the decrease in birefringence (I) and the increase in isometric twitch tension (P) (Phase retardation at rest 110 mμ, equilibrium length, 7 C, end-stimulation)
Below
The micro pipette (M) was inserted close to the edge of the illuminated part of the muscle fibre (S) indicates the position of the stimulating electrode Due to the movement of the fibre the electrode left the fibre at the time indicated by the arrow
The time constant T of the amplifier for recording of light intensity changes was 1.5 sec. The distortion by T was negligible

With transverse stimulation at 12 C the latency of the change in birefringence was 1 1 ± 0 2 msec shorter than the latency of tension stimulating the end of the fibre the difference was 3 3 ± 0 5 msec

At both 7 and 12 C the rise time of the change in birefringence was shorter than the rise time of tension (Fig 9) half as long to peak and 30 per cent shorter to half peak with transverse stimulation (Table II) The difference in rise time between birefringence and tension was not due to the time constant of the amplifier used for recording of light intensity changes (Fig 9)

The rate at which birefringence and tension returned to the pre twitch level was the same When relaxation was complete birefringence increased for about 200 msec by 1 6 per cent of the birefringence of the resting fibre (corrected for the distortion caused by the lower limiting frequency of the amplifier six experiments with a mean maximum decrease in birefringence of 7 per cent)

2 *The action potential the decrease in birefringence and twitch tension simultaneously* The fibre was stimulated at one end and the intracell

Table II Time relationship between the change in birefringence and the twitch tension

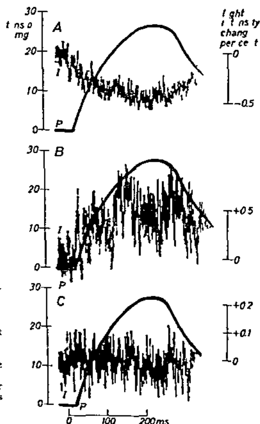
Latencies time to half peak and to peak amplitude

	Transverse stimulation				End stimulation			
	7 C		1? C		7 C		12 C	
	t_1	τ_1	t_1	τ_1	t_1	τ_1	t_1	τ_1
S E M	8 4 ± 0 4	11 5 ± 0 6	5 7 ± 0 4	6 8 ± 0 3	10 6 ± 0 5	15 3 ± 0 7	8.2 ± 0 5	11 4 ± 0 7
$t_1 - \tau_1$	3 2 ± 0.5		1 1 ± 0 2		4 7 ± 0 7		3 3 ± 0 5	
No of exper ment	14		10		8		9	
	t_2	τ_2	t_2	τ_2	t_2	τ_2	t_2	τ_2
S E M	19 1 ± 1 4	32 6 ± 2 3	17 7 ± 1 2	23 7 ± 1 3	23 6 ± 1 9	35 0 ± 3 1	18 6 ± 0 8	26 0 ± 1 5
No of exp riments	10		9		5		7	
	t_3	τ_3	t_3	τ_3	t_3	τ_3	t	τ_3
S E. M	49 1 ± 6 2	96 0 ± 6 8	29 7 ± 3 5	60 8 ± 4 3	64 2 ± 4 5	96 4 ± 11 0	47 6 ± 2 9	64 3 ± 4 6
No of exp riment	10		9		5		7	
Sar omer length	2 44		2 46		2 50		2.53	

t_1 L tency of 1 cre n b r fringence
τ_1 Lat ncy of th posit e ten on
t_2 Time from the timulus to h lf the maximum decrease n b efringenc
τ_2 T me f om the stimulus to half the peak tens on.
t_3 T me from th timulus to the maximum decre se n bi efringence.
τ_3 T me from the stimulus to the peak tens on

posite direction with the Polaroids crossed and parallel and there was no change in light intensity in non polarized light Except for the faster initial rise and for a slight increase in phase retardation at the end of the twitch the time courses of the decrease in phase retardation and of the increase in tension were similar

Latency and rate of rise With transverse stimulation at 7 C the first change in birefringence occurred 8 4 ± 0 4 msec after the stimulus the onset of tension development was 3 2 ± 0 5 msec later (sarcomere length 2 44 ± 0 04 μ) When one fibre end was stimulated so that the activity was conducted to the region in which birefringence was measured birefringence started to decrease 10 6 ± 0 5 msec after the stimulus and tension started to develop 4 7 ± 0 7 msecs later (7 C sarcomere length 2 4 — 2 5 μ Table II)



Fg 12 Th hang l ht nt ns ty and n t ns n t h gh t l t etch
A d P la ds B p llel Po- l d
C n n p l iz d l ght
Th 0 5 p cent d ea n l ht in ten ty c pond d to a 0 4 p ent d r as n b fring nc The ph t dat n t est wa 165 m (S m l gth 3 8 μ 7 C t ansverse timul ti n)
Th t m c n tant T f th mpl fier f co di g f l gh nt n ty han es was 1 5

T bl III Ch g l ht tens ty and t nsio d tu tch and t t us

Temp C	Stimul f eq ency p	Ch g l ght t ns ty			Tens n		
		I wi h pe en	I tan per ent	$\frac{I_{tetanus}}{I \quad ch}$	P wi h mg	P tanus mg	$\frac{P_{etanus}}{P \quad h}$
1?	15	12 0	1 0	12	260	371	143
12	40	6 0	6 0	100	193	7	133
1?	20	11 5	11 5	100	33	408	1 3
12	0	1 5	1 0	96	300	400	133
7	40	5	5 0	91	3 4	400	1 3
7	40	3 7	4 0	108	1 9	215	135

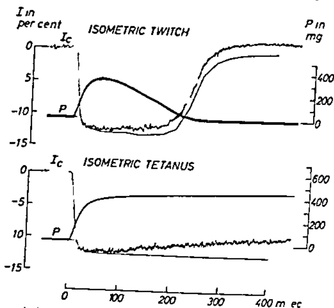


Fig. 13. Decrease in light intensity (I) (crossed Polaroids) and increase in tension (P) during an isometric twitch and a tetanic contraction.

Note that the decrease in birefringence is the same in the twitch and the tetanus while the tetanus tension is 1.3 times the twitch tension. (Phase retardation at rest 260 mμ; sarcomere length 2.6 μ; 12° C; transverse stimulation.)

The time constant T of the amplifier for recording of light intensity changes was 1.5 sec. The thin lines give the time course corrected for the distortion by T.

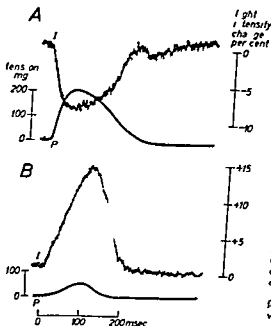


Fig. 14. (A) Decrease in light intensity (I) and increase in twitch tension (P) in the undamaged fibre. (B) Increase in light intensity (I) and 70 per cent reduced twitch tension after damage had developed. (Eq. 1b; sarcomere length [illegible] 7° C; end-stimulation.)

The time constant T of the amplifier for recording of light intensity changes was 1.5 sec.

An example of the change in tension and birefringence in a single fibre is shown in Fig 11 A and the average curve of 19 experiments in Fig 11 B

At sarcomere lengths of 3 6 and 3 8 μ (64 and 73 per cent stretch) the decrease in birefringence during twitch was still visible and amounted to 0 8 and 0 4 per cent respectively (10 experiments) (Fig 12)

4 *Comparison between the decrease in birefringence during twitch and tetanus* In six fibres birefringence and tension were recorded during twitch and tetanus at 7 and 12 C The fibres were at equilibrium length or slightly above it The decrease in light intensity was the same during twitch and tetanus tension was 30 per cent greater during tetanus than during twitch (Fig 13 Table III)

5 *Deteriorating fibre* Recordings were never taken before one hour after the fibre was mounted in the chamber and the fibre usually remained in the same condition for several hours When a fibre was deteriorating the birefringence increased at rest (BUCHTHAL and KNAPPEIS 1938) and the changes in birefringence often were altered before twitch tension decreased Changes in light intensity during contraction might suddenly reverse as if birefringence increased by as much as 15 per cent (Fig 14)

Discussion

During isometric contraction of whole muscle even if only a small part is illuminated and the muscle is compressed it is impossible to prevent changes in the light path by small displacements of a few fibres nor because the degree of stretch varies in different fibres (BUCHTHAL KAISER and ROSENFALCK 1951) can the relationship be established between the initial degree of stretch and the change in birefringence Measurement of birefringence and tension in the isolated fibre avoids these sources of error In single fibres BUCHTHAL and KNAPPEIS (1939) found from the displacement of the interference fringes a fall in birefringence during contraction but their method did not permit the time course of the change to be followed With light intensity changes as a measure of birefringence the time course can be followed In single fibres with a phase difference smaller than 3 7 radians the relation between birefringence and light intensity is simple and unambiguous

We have found that birefringence decreases before positive tension develops in single muscle fibre during isometric twitch In agreement with von Muralt s findings in whole muscle (1932) birefringence reaches its minimum before the peak of twitch tension From experiments on whole sartorius VON MURALT (1933) gives an example of a 27 per cent decrease in phase retardation BOZLER and COTTRELL (1937) found in whole sartorius a 30 per cent decrease They measured continuously the displacement of interference fringes representing changes in maximum birefringence changes in birefringence measured by changes in light intensity rather are changes in average birefringence This may explain why the decrease in birefringence in single fibres only averaged 9 per cent The

change in birefringence found by von Muralt (1932) in whole muscle had a more complex course than in the isolated fibre; it had two minima, the first *slightly before the peak of tension and the second during relaxation. In isolated* fibres such double peak changes in phase retardation were not observed unless the fibre twisted during contraction.

The birefringence of the muscle fibre is the result of crystalline birefringence, form birefringence (Stübel 1923, Noll und Weber 1934) and possibly polarization phenomena in narrow slits[1] (Hönl und Zimmer 1953). The decrease in birefringence during contraction may be due to an increase in the number of cross links between minute structural elements or to a displacement of fluid within the fibre (A. F. Huxley 1957). In polystyrene an increase in the number of cross links may account for as much as 65 per cent decrease in birefringence (Gurnee 1954). A formation of cross-links would diminish both crystalline and form birefringence.

The increase in torsional stiffness during isometric contraction has been interpreted as being due to the formation of cross links (Sten-Knudsen 1953). This phenomenon has a time course similar to that of birefringence in the initial phase of contraction. Torsional stiffness increases and birefringence decreases before positive tension develops and both reach peak before the peak of tension. The decrease in birefringence was the same in twitch and tetanus whereas tension was 20 to 40 per cent greater in tetanus. Similarly during transition from twitch to tetanus torsional stiffness increased less than tension.

The early onset of the change in birefringence, its steep fall and the identical change in twitch and *tetanus indicate that the decrease in* birefringence may be related to the process of activation (active state, A. V. Hill 1949, 1953 b).

During twitch of single fibres at higher initial lengths birefringence changed less as did tension. The development of tension in contraction has been attributed to the interaction between A and I filaments in the zone of overlap (H. E. Huxley and Hanson 1954, A. F. Huxley and Niedergerke 1954, Hanson and H. E. Huxley 1955, A. F. Huxley 1957). It was therefore near at hand to localize the site of the formation of cross links to the zone of overlap. However, in a fibre stretched to a degree that there is no longer any overlap a formation of cross links between A and I filaments cannot account for the decrease in birefringence. Nor can the change in polarized light at long lengths be attributed to a decrease in fibre diameter due to passive stretch of the middle part of the fibre when its ends shorten, since non polarized light remained unchanged during the twitch.

A displacement of water within the fibre may alter the form birefringence and the slit polarization since both are attributable to differences in refractive indexes of the structural components. In addition, a shift of water affects the fraction of volume occupied by the minute structural elements. Refractive

[1] This possibility was suggested by D. R. Later

indexes and volume fractions have hitherto not been determined in the living fibre during contraction This makes the evaluation of the decrease in birefringence in terms of fluid displacement rather uncertain

In the deteriorating fibre birefringence may remain unchanged or even increase during a twitch This may be the result of local contraction of the undamaged portion of the fibre pulling more birefringent material into the light path at the expense of the injured portion These irregularities may occur before twitch tension is reduced but the subsequent decreas in tension indicates that the fibre is deteriorating

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W are d bt d t Pr f sor A F HUXLEY F R S f permiss on to u h s calcul t ons of th rel t on between l ght nte ty a d b ref ing c f the ell pt cal fib e

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From The Neurophysiological Laboratory, Anatomical Institute, University of Oslo, Norway

Slow and Fast Muscle Fibres in the Atlantic Hagfish

(Myxine Glutinosa)

By

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Abstract

ANDERSEN, P., J. K. S. JANSEN and Y. LØYNING. *Slow and fast muscle fibres in the Atlantic hagfish.* Acta physiol. scand. 1963. *57.* 167—179. — The two histologically different types of muscle fibres in the parietal muscle of the hagfish have been studied by means of internal microelectrodes and by recording the mechanical response after stimulation of their motor nerves. The one type of fibre gives rapid twitch contraction of about 150 msec duration while those of the other type last more than 500 msec. Accordingly the muscle fibres are called fast and slow, respectively. The resting membrane potentials of the fast fibres were 74.6 mV (S. E. 0.3), that of the slow fibres 46 mV (S. E. 0.5). Indirect stimulation of the fast fibres evoked a characteristic focal end plate potential in one end of the fibre. The end plate potential gave rise to a conducted action potential with overshoot. The fast fibres appear to be innervated with only one motor axon each. In the slow fibres distributed junction potentials were obtained on indirect stimulation. The time course of the junction potentials varied partly on account of local spike activity. On repetitive stimulation of slow fibres a plateau depolarization was reached which only moderately exceeded the peak of a single junction potential. The slow fibres are usually innervated with two motor axons reaching the fibre from each end.

Since the description of two functionally different types of striated muscle fibre in the frog by KUFFLER and VAUGHAN WILLIAMS (1953a, b) the slow fibre system has attracted considerable interest. Its presence has been established in all groups of vertebrates except mammals (see PEACHEY (1961) for a review). However, there appears to be considerable differences in the functional properties of the various slow muscles which have been studied. Even the classification of one type of fibre as fast or slow may sometimes be difficult. Therefore

an investigation of the properties of the parietal muscle of the hagfish was considered of interest. The hagfish is one of the most primitive vertebrates and the existence of two histologically different types of muscle fibre has been known since the work of MAURER (1894) and COLE (1907). Preliminary observations on these muscle fibres have been reported earlier (JANSEN and ANDERSEN 1960).

In the parietal muscle of the hagfish the muscle fibres are regularly arranged longitudinally in myotomes. Each fibre extends throughout the entire length of the myotome, a distance of about 3 mm. The motor nerves run in the myocommata separating the myotomes. The two types of muscle fibre can be distinguished by their size and histological appearance. The thick fibres have a motor end plate at the end of the fibre. The thin fibres, on the other hand, are usually supplied by two motor nerve fibres, one from each of the adjacent myocommata. These nerve fibres run along the entire length of the muscle fibre and show numerous synaptic expansions in their course (JANSEN, ANDERSEN and JANSEN, unpublished).

Methods

The experiments were carried out on the parietal muscle of adult hagfishes (*Myxine glutinosa*). The animals were decapitated and about 2 cm long pieces cut out of the body wall muscles in the middle part of the animal. Particularly the ventral part of the muscle consisting of a fairly thin sheet of muscle fibres was used, after removal of the oblique superficial muscle. The preparation was kept in a bath of sea water or an artificial salt solution between two clamps. The solution used contained NaCl (5[illegible]0 mM), KCl (11 mM), $MgCl_2$ (16 mM) and $CaCl_2$ (5 mM). No particular difference was observed between preparations in sea water or the artificial solution in the present experiments. The preparation was dissected by means of small forcepses in transmitted light under a dissection microscope until a limited number of muscle fibres remained in the area to be studied. The two types of muscle fibre are clearly distinguishable under the microscope. The experiments were all performed at room temperature (18—20° C).

The recording electrodes were glass micropipettes filled with 3 M KCl. Their resistances measured 10—[illegible]0 MΩ. The recording system was conventional with a cathode follower input stage, the grid current of which was less than $10^{-1[illegible]}$ A. After D.C. amplification the responses were displayed on a dual beam oscilloscope. One beam was kept as a zero mV reference line. Drift in the system was occasionally detected after retraction of the electrode and the observation discarded. The rise time from 10 to 90 per cent of an applied step voltage for the whole system with a 20 MΩ electrode was approximately 220 μsec.

The motor nerves were stimulated by two 0.1 mm insulated silver wires kept close together by a varnish and applied to the myocommata. Rectangular pulses of required intensity and interval were supplied by two stimulators. The pulse duration was usually 0.1 to 0.2 msec.

Contraction of a group of 5 to 10 muscle fibres was recorded by a RCA 5734 transducer valve, the anode of which was extended by a glass tube to [illegible]0 mm. A sharpened fine steel needle was fastened to the end of the glass rod. This tip was inserted into the connective tissue of the myocomma close to the attachment of the muscle fibre. Attached to this lever the muscle fibres were allowed to shorten less than 5 [illegible] of their initial length during the contraction.

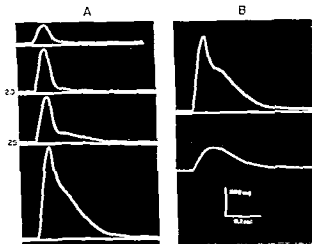


Fig 1 Contract on f a mall gr p of mi ed fast and low muscl fib es A. Effect f increa ing st m ulus tre gth Intens ty f st mulat n in b trary units t th l ft of each r c d. N t appearan of slow c mpo nt w th tr g hock B Top re o d trol esp nse. Lower rec rd aft r a peri od of epet t st mul t n. Fast c mponent blocked

Results

The hagfish parietal muscle offers a considerable advantage in that the two types of muscle fibres can be readily distinguished under the dissecting microscope The one type is thicker and of a glassy appearance the other is thin granular and of a lightly brownish colour The thick fibres predominate on the peritoneal surface of the preparation while the superficial layer of fibres on the lateral side are mainly of the second variety (MAURER 1894) When the motor nerves in the intersegmental septa are stimulated the contraction of the two types of fibres is easily observed That of the thick fibres is rapid and twitch like The thin fibres on the other hand contract more slowly Their prolonged relaxation is particularly evident Usually the two kinds of fibre contract simultaneously Then the relaxation is seen to consist of a rapid and a later slow phase Under various experimental conditions and by careful adjustment of the intensity of the stimulus one can observe contractions consisting mainly or exclusively of one or the other type In the following these fibres will be called fast and slow fibres respectively

Mechanical responses The tension changes following single shock stimulation of the motor nerves in a mixed group of muscle fibres in the thin ventral part of the parietal muscle are illustrated in Fig 1 A slightly suprathreshold stimulus elicited a twitch contraction of about 150 msec duration and a peak time of 60—80 msec (Fig 1A 1 15) A moderate increase in stimulus intensity made a second slower phase increasingly apparent The duration of the contraction was now more than 400 msec and the slow relaxation of the second component was clearly seen The appearance of the second component in the tension record was always correlated with visible contractions in the slow muscle fibres

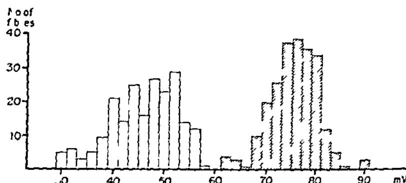


Fig 2 Histogram of resting membrane potential of fast and slow fibres Membrane potential along abscissa and number of fibres in each group along the ordinate Slow fibres open columns, fast fibres cross hatched No overlap between the two groups

The twitch component of the contraction was more susceptible to fatigue than was the slow contraction Isolated contractions of the slow fibres could then be observed as in Fig 1B The top record shows the control consisting of a rapid and a slow component After a period of repetitive stimulation only slow fibres were contracting and the rapid component was no longer present in the tension record (Fig 1B lower record) The time to peak of the slow contraction was about 120 msec which approximately corresponds to the total duration of the twitch in the thick muscle fibres

These observations suggest that the two kinds of muscle fibre differ considerably in their functional properties and in the following experiments a comparison of the neuro-muscular transmission and electrical activity of the fibres has been made

Resting membrane potentials The resting potentials have been measured with an internal microelectrode in a large number of twitch and tonic muscle fibres. Fig 2 shows the values obtained from more than 200 fibres of each variety obtained in 13 different specimens The experiments were all performed at room temperature (18—20 C) and the animals were used as soon after catching as possible Only when the penetration of the fibre was easy and the full resting potential was established immediately and remained stable the value obtained was included in the series The zero level was checked after retraction of the electrode and if appreciable drift had taken place the observation was excluded

The mean value of the resting potential of the fast fibres in Fig 2 was 74 6 mV (S E 0 3) that of the slow fibres was 46 mV (S E 0 5) Thus the two types of fibre appear to be clearly distinguishable by their resting potential level No overlap was observed between the two groups In any one experiment the resting potentials of fast muscle fibres showed only small variations The values obtained for the slow fibres were less uniform That is probably because these fibres on account of their smaller size are more likely to be injured by the

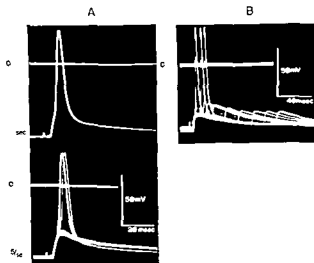


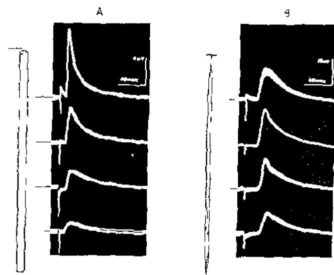
Fig. 3. Action potentials of fast muscle fibres after stimulation of motor nerves. A. Upper record: shocks at sec intervals, 5 superimposed sweeps. Note step on rising phase. Lower record: partial block of transmission on 5/sec stimulation. B. After a period of repetitive stimulation which caused complete block to single shock activation. Paired shocks at various intervals. Summation of e.p.p. and action potentials at shortest intervals.

microelectrode. They proved to be more difficult to penetrate and would sometimes contract when only touched by the microelectrode. The greater difficulty of penetration may partly be due to the dense meshwork of capillaries surrounding these fibres (JANSEN, ANDERSEN and JANSEN, unpublished).

An important question is whether this difference in resting potentials of the two kinds of fibre here observed is a genuine difference or merely due to a difference in the susceptibility to injury during penetration. This problem cannot be answered conclusively by the present experiment. It appears entirely possible that a greater injury to the slow fibres may partly account for the considerable difference between the two groups. However, in injured fibres the resting potential will usually decline progressively over a period of time. In the slow fibres of the hagfish a resting potential above 60 mV was never observed even immediately after penetration. Furthermore, one slow fibre has often been repenetrated a number of times without appreciable decline in resting potential level. For these reasons it does appear likely that there is a genuine difference in the level of resting membrane potential of the fast and slow muscle fibres. A similar but less marked difference has been found for corresponding fibres in other vertebrates (KUFFLER and VAUGHAN WILLIAMS, 1953a; BARETS, 1961).

Neuromuscular transmission in fast muscle fibres

Electrical stimulation of the motor nerves running in the myocommata regularly elicited an action potential in the fast muscle fibres on both sides of the myocomma. The action potential was conducted without decrement along the whole length of the fibres. Except when the fibres were clearly injured and partly depolarized, the spike always showed an overshoot of 30—40 mV



F g 4 D strib t on of e p p and j p s along the two kinds f fibre A E p p at diff r nt sites f fa t fibre nd cat d by draw ng of th fibre Ne omusc lar block by cu ari e 10 M t r nerve t rmina i g o pper nd of fib e timulated B J p s at different tes of slow fibr after t mul tion of nerv ent ring from upper end 5 s ceps s perim posed in ach record i A and B

Much like the action potential of the fast muscle fibre of the frog (NASTUK and HODGKIN 1950) the repolarization of the membrane occurred in two phases a rapid phase followed by a much slower one The duration of the rapid phase of the action potential was from 4 to 6 msec After hyperpolarization was only seen in depolarized fibres

When recorded within one mm from the end of the fibre an initial slower phase could usually be seen on the rising phase of the spike (Fig 3A) After a period of repetitive stimulation at moderate frequencies (5—20 per sec) the action potential was blocked and the much slower initial potential was seen in isolation (Fig 3A lower record) From its characteristics (time course distribution summation) it can safely be interpreted as the electrotonically conducted end plate potential (e p p) In Fig 3B obtained after a period of repetitive stimulation there was complete block of transmission Only e p p s were elicited by single shocks to the nerve On reducing the interval between a pair of shocks the e p p s were summated and spikes were initiated at the greater depolarizations In freshly dissected preparations the e p p was always great enough to elicit action potentials After some hours of experimentation various types and degrees of block would develop in many fibres As shown in Fig 3 repetitive stimulation was particularly effective in creating blocks even at low frequencies

Distribution of e p p The neuromuscular transmission in the fast fibres can be blocked by curarine in concentrations like those producing blocks in other vertebrate muscles In the curarized preparation the distribution of the e p p along the length of the fibre can be studied without the complications of action potentials A typical series of records are shown in Fig 4A The e p p was always largest close to the septum in which the motor nerves were stimulated

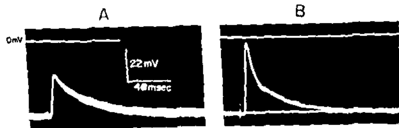


Fig. 5. J.p.s of two different slow fibres (A and B) after indirect stimulation. Each record consists of 5 superimposed sweeps. In B one shock was just below threshold for the fibre. The other was slightly above threshold. No further increase in j.p. on increasing stimulus intensity.

The rapid reduction in amplitude, increasing duration of rising phase and progressive slowing of rate of repolarization is characteristic for the e.p.p. as the distance from the active focus is increased (FATT and KATZ 1951). This observation agrees with the histological finding that the endplates of the fast fibres are localized close to the end of the parietal muscle fibres (JANSEN ANDERSEN and JANSEN unpublished).

In methylenblue sections the motor nerves in a myocomma are clearly seen to terminate on muscle fibres on both sides of the myocomma (RETZIUS 1892; JANSEN ANDERSEN and JANSEN unpublished). Therefore the question arises whether one fast muscle fibre is supplied with end plates at both or merely at one end. For technical reasons quantitative information on this problem is not easily obtained by traditional histological methods. In the present experiments attempts were made to activate fast muscle fibres by stimulation of the motor nerves in both neighbouring myocommata. Both stimuli elicited contractions in the same myotome. But on penetration of a large number of fast muscle fibres it appeared that each fibre was activated either from the anterior or the posterior end. By recording close to the end of the fibre end plate and end plate distant records could be distinguished. No observation was made indicating activation of the fast muscle fibres from both ends. Thus with the present methods the fast muscle fibres appear to be innervated by a single axon terminating in an end plate localized at the anterior or posterior end of the muscle fibre.

Neuromuscular transmission in slow fibres

The electrical activity of the slow muscle fibres in response to stimulation of their motor nerves was characteristically different from that of the fast fibres. The typical response is shown in Fig. 5A. It consisted of a fairly rapid depolarization with a rising phase of 5—10 msec in different fibres followed by a much slower and nearly exponential repolarization. On account of its similarity with corresponding potentials particularly in Crustacea (HOYLE and WIERSMA 1958) this slow fibre response will be called a junction potential (j.p.). The amplitude of the j.p. could vary considerably (from about 5 to more than 30 mV) in different fibres and could thus very nearly reach zero membrane potential at peak depolarization. In any one fibre the amplitude usually remained constant as long as the resting potential was unchanged

However, many slow fibres were progressively injured by the microelectrode in the contracting fibre and the j. p. would then decrease with the level of resting membrane potential. In depolarized fibres the j. p. was often followed by a prolonged period of hyperpolarization.

Time course of j. p. The time course of repolarization could vary considerably in different fibres. The time constants of decay of the j. p. could vary by more than a factor of three, from about 20 to 60 msec even when the decay was reasonably well described by a simple exponential. Quite often, however, there was a definite change in the rate of repolarization of the j. p. after about 15 msec. An initial rapid phase was followed by a slower late repolarization (Fig. 5B). This was particularly seen when the response was large and occurred usually without any apparent discontinuity on the rising phase of the j. p., but sometimes in presumably injured and partly depolarized fibres a small change in rate of rise of the j. p. could be observed just before peak depolarization (Fig. 6B). Similar irregularities in the time course of membrane potential changes have been interpreted as abortive action potential in slow fibre membrane (HOYLE and WIERSMA 1958, TAKEUCHI 1959) and it appears reasonable to interpret the present observations as such.

Relation to stimulus intensity. When the strength of the stimulus to the myocomma containing the motor nerves was increased, the j. p. always occurred at a particular threshold and was then of full size (Fig. 5B). Further increase in the intensity of the stimulus to up to 5 times threshold did not change the amplitude or time course of the response. This is in agreement with the histological observation of only one motor nerve from each myocomma to each slow muscle fibre (JANSEN, ANDERSEN and JANSEN, unpublished). Occasionally a fluctuation of about 10 per cent was observed in consecutive j. p. elicited at 2 sec intervals, but this fluctuation had no correlation with changes in stimulus intensity. After a period of rest the first j. p. was up to 30 per cent larger than the later ones which remained of fairly constant amplitude.

Distribution of j. p. s. As was to be expected from the mode of innervation of the two kinds of muscle fibre the distribution of the j. p. s. along the fibre was entirely different from that of the e. p. p. The j. p. s. were of comparable amplitude and time course throughout the entire fibre and the variations in size and shape that occurred did not bear any relation to the part of fibre from which they were recorded. Thus in the series of records shown in Fig. 4B the latency of the j. p. increased as the distance from the stimulated septum was increased, but the amplitude of the j. p. s. did not show any consistent change with increasing distance. This is in clear contrast to the e. p. p. (Fig. 4A) which clearly originated at the innervated end and spread passively along the fibre.

Summation of j. p. s. The effect of preceding activity is of particular interest in slow fibre neuro-muscular transmission. The j. p. s. of many Crustacean muscle fibres are largely faciliated by repetitive activity (HOYLE and WIERSMA



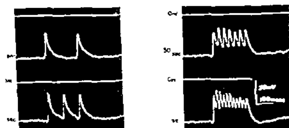


Fig 6 S mm t on of j p A and B p ed l ocks at rying interval In B mall d sco t nu ty n n g phase marked by an arrow C Tra ns of t m l of creas g frequencies ndi cated t the left of ach e ord

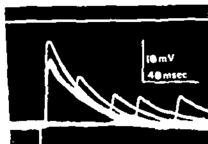


Fig 7 Dual act at n of 1 w fibr Nerve fibres from th anteri and th posteri r r yocomma timulated at d fferent intervals.

1958) whereas the frog j p s are not appreciably influenced by preceding activity (KUFFLER and VAUGHAN WILLIAMS 1953a) In hagfish slow muscle fibres a second j p elicited soon after the first would sum with the preceding one and thus add to the depolarization (Fig 6A B) At short intervals however the second j p was usually considerably depressed so that the maximal depolariza tion was not more than 150 per cent of a single j p Even so during summation of j p s the membrane potential of the fibre was often reversed Only one slow fibre with an appreciable facil tation of the j p at short intervals has been found in these experiments Besides the reduction in amplitude a second j p at short intervals usually showed an increased rate of repolarization (Fig 6B) As expected from these observations summation of j p s is not very effective in the slow fibres In the experiment of Fig 6C the motor nerves were stimulated by a train of shocks of 0 2 sec duration and the effect of increasing the frequency of stimulation was studied At the higher frequencies (30 sec 50/sec) the fibre was kept depolarized during the train but the "ceiling depolarization was reached already by the first j p

Dual activation The slow muscle fibres are innervated by two motor axons one from each of the neighbouring myocommata The j p s elicited in a fibre by *stimulation of the two axons at different time intervals are illustrated in* Fig 7 The records were obtained from the middle of the muscle fibre and characteristically the one j p was considerably greater than the other When a fibre was penetrated closer to its ends the j p evoked from the nearest myocommma was not necessarily the largest The experiment of Fig 7 clearly shows that the two j p s were summated at all intervals As must be expected the second j p was somewhat smaller when elicited during the depolarization of the first The records suggest however that the ceiling depolarization of *the fibre will be appreciably higher during repetitive activity in both its motor* axons than that produced by activity in either one alone

Relation between contraction and electrical activity By visual observation of the impaled fibre in the dissecting microscope it was seen that contraction of the fast muscle fibres was always associated with an action potential The slow fibres on the other hand was clearly contracting when a smooth j p was recorded across the fibre membrane The threshold for electrical and mechanical response of the fibre was identical This does not exclude the possibility that localized spike activity was taking place in other parts of the fibre membrane at the same time However the frequent observation of simple j p s in contracting slow fibres favours the conclusion that spike activity does not play an essential part in the activation of the contractile mechanism of these fibres

Discussion

The existence of two kinds of muscle fibre with different functional properties in the parietal muscle of the hagfish appears to be established by the present observations Slow muscle fibres have been demonstrated in the other major groups of vertebrates as well such as amphibia (KUFFLER and VAUGHAN WILLIAMS 1953a) birds (GINSBORG 1960a b) and fishes (TAKEUCHI 1959 BARETS 1961) In all these vertebrates the slow muscle fibres are thinner than the twitch fibres and their motor nerve terminals are distributed along the entire length of the muscle fibre Only in mammals do these fibres appear to be lacking although certain observations suggest that some of the intrafusal fibres of the muscle spindles may be of this type (HUNT and KUFFLER 1951 BOYD 1961)

However the slow fibre systems of the different vertebrates are by no means a uniform group with regard to their functional properties and the slow fibres of the hagfish appear to be characteristically different from the others in certain important respects Thus in frog slow fibres which have been particularly well investigated only very small or no contraction was elicited by a single stimulus to the motor nerve (KUFFLER and VAUGHAN WILLIAMS 1953b) The j p s were summated without any change of the second j p and during tetanic stimulation the plateau of maintained depolarization might be two or three

times that of a single j p depending upon the frequency of stimulation (KUFFLER and VAUGHAN WILLIAMS 1953a) Similarly in the red muscles of the snakefish a level of depolarization was reached during repetitive indirect activation which might be 5 times that of isolated j p and determined by the stimulus frequency (TAKEUCHI 1959) A certain amount of facilitation of the later responses in the train apparently took place in the snakefish muscle (TAKEUCHI 1959 Fig 7) In the hagfish slow fibres on the other hand brisk contractions were evoked also by single stimuli the second j p was usually depressed at short interval and summation was not very effective The plateau depolarization during a train of stimuli did usually not appreciably exceed the peak value of a single j p (Fig 6C)

Furthermore in the frog action potentials are not produced by the slow fibre membrane (BURKE and GINSBORG 1956) and neither in the snakefish following indirect stimulation (TAKEUCHI 1959) Again the irregularities in the time course of the j p s in the hagfish (Fig 5B 6B) suggested that local spike activity was fairly common in these fibres The additional observation of slow fibre contractions elicited by direct electrical stimulation in curarized preparations may be mentioned in this connection It is unknown however which part the abortive action potentials may play on the activation of the contractile mechanism of the hagfish slow fibres

All the other vertebrate slow muscle fibres are innervated by several motor axons Thus when the motor nerve is stimulated by increasingly stronger shocks the j p can be seen to increase in discrete steps as new axons are recruited In contrast the hagfish slow fibres usually receive only one motor axon from each septum so that when a group of motor axons was stimulated in the myocomma the j p of a particular slow fibre always appeared full sized immediately the threshold was reached (Fig 6)

All these observations go to show that the hagfish slow muscle fibres do not possess the possibilities for grading the mechanical response that exist in other slow fibre systems If the degree of mechanical response of hagfish slow fibres is determined by the depolarization of the fibres as seems to be the case for frog slow muscles (KUFFLER and VAUGHAN WILLIAMS 1953b) the contraction will be graded only to a limited extent by the frequency of stimulation In this respect the hagfish slow fibres are more like twitch fibres in other vertebrates Additional activation of the second motor axon of the slow fibre on the other hand will add to the depolarization (Fig 7) and presumably to the contraction as well

Compared with fast muscle fibres in other fishes those of the hagfish possess a particularly effective spike mechanism Most of the fibres receive only one motor axon terminating on the end of the muscle fibre The white muscle fibres of the snakefish (TAKEUCHI 1959) on the other hand are polyneuronally innervated with distributed terminals and the action potentials rarely showed an overshoot Furthermore during repetitive activation only one action

potential was elicited in these fibres but they were kept depolarized by the j p s and the contraction maintained for the duration of stimulation Rather similar properties appear to exist in the deep lateral muscle of the tench (BARETS 1961) The hagfish fast muscles are more like the fast muscles of for instance the frog in which the contractile mechanism is activated by action potentials only The deep lateral muscle of the catfish appear to have similar properties (BARETS 1961) The suggestion of TAKEUCHI (1959) that the action potential mechanism is of less importance in the muscles of the phylogenetically lower vertebrates are not supported by the present observations in hagfish muscles

From the point of view of ionic composition the hagfish muscle presents certain interesting problems The Myxinoids are the only vertebrates with an extracellular fluid similar to sea water in ionic content (ROBERTSON 1960, BELLAMY and CHESTER JONES 1961) The intracellular content of sodium of the parietal muscle fibres is as high as 152 mM per kg fibre water (BELLAMY and CHESTER JONES 1961) The ratio of intracellular to extracellular potassium is about 17 If this potassium distribution depends upon the resting membrane potential of the fast muscle fibres the membrane potential can be calculated to 72 mV at 20 C a value which is close to the observed mean of 75 mV (Fig 2) If the resting potential of the slow fibres (46 mV Fig 2) similarly are determined by the potassium distribution the estimated internal potassium concentration is then only about 73 mM per kg If therefore sodium is evenly distributed between the two kinds of fibre the internal concentration of potassium in the slow fibre would be less than that of sodium A high sodium content has been reported for slow muscles of Pecten as well (POTTS 1958)

As regards the functional significance of the slow fibre system of the vertebrates in their motor activities it is generally believed that they are mainly concerned with postural contractions (KUFFLER and VAUGHAN WILLIAMS 1953b PEACHEY 1961) For aquatic animals however postural problems must be of minor importance An alternative function of the slow system in fish may be suggested by the observations of oxygen requirements in fish The available evidence shows that fish can only use their muscle fully for short periods of time on account of the limited oxygen supply from their respiratory organs (GRAY 1953) One can then imagine that the fish employs its powerful and numerous fast fibres of the parietal muscle mainly in situations when maximal and rapid propulsive movements are required The slow fibres on the other hand may be sufficiently powerful to carry out the slower movements of ordinary life The mainly peripheral localization of the slow fibres which gives them a greater momentum with respect to the spinal column and the rich vascular supply of slow fibres should be noted in this connection Both these last mentioned features appear to exist in the lateral muscle of fish (BARETS 1961) The present suggestion of the significance of the two types of fibre is entirely hypothetical and observations on their normal and reflex activation are needed in order to elucidate the problem

We a e ind bted t Professor BJORN FOYN D re tor of the B ological stati n DROBAK f r g n ous pply of hagfi h Th help of lectr engineer G I ILLEH IL is gratefull ckno l dged.

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Nervous and Local Chemical Control of Pre-Capillary Sphincters in Skeletal Muscle as Measured by Changes in Filtration Coefficient

By

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Rece d 30 June 19(?)

Abstract

Cobbold A B Folkow I Kjellmer and S Mellander *Nervous and local chemical control of pre capillary sphincters in skeletal muscle as measured by changes in filtration coefficient* Acta physiol scand 1963 57 180—192 —The influence of nervous and local chemical factors on the pre-capillary sphincter section of the muscle vascular bed has been studied in terms of changes in the filtration coefficient (CFC) The results suggest that the vasoconstrictor fibres beside their powerful influence on the resistance and capacitance vessels affect also the pre capillary sphincters which determine the number of capillaries open to flow However the influence exerted by the centrally controlled vasoconstrictor fibres on the pre capillary sphincters in the skeletal muscles is rapidly overcome by vasodilator metabolites accumulating when flow is reduced or metabolism is increased Such local metabolic factors exert an especially powerful control of the sphincter section as compared with the neurogenic influence Thus CFC in the skeletal muscles tends to rise at any decrease of blood flow/tissue metabolism ratio e g from resting values around 0 015 up to 0 04—0 05 during muscular exercise an increase also occurs when flow is reduced Flow reduction following mechanical arterial obstruction induces a relatively greater CFC increase than when flow is reduced by the constrictor fibres which unmasks their weak but still significant influence on the pre capillary sphincter section

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From a functional point of view the vascular bed of a tissue may be considered as being composed of a set of specialized series coupled sections (FOLKOW 1959) in which the key points of the vascular bed the true capillaries are situated between pre-capillary resistance vessels and post capillary resistance vessels Further there are capacitance vessels which are confined mainly to the venous side of the circulation So far as the true capillary bed is concerned a considerable measure of control must be exercised predominantly via the terminal portions of the pre capillary resistance section *i e* the sphincter section or — as they are often called — the pre capillary sphincters Contraction of the smooth muscles can produce a complete closure of the capillary lumen and therefore these sphincters form a functionally specialized and separate section in their own right as they will determine the number of capillaries that are open to blood flow and hence to effective transcapillary exchange at any given moment The control of the resistance vessels has been extensively investigated with quantitative methods (FOLKOW 1955 1959 MELLANDER 1960) as has that of the capacitance vessels (MELLANDER 1960) The functional behaviour of the pre capillary sphincters may be inferred from the determination of the capillary filtration coefficient (CFC) CFC is defined as the quantity of fluid filtered through the capillary surface in ml/min/100 g tissue/mm Hg pressure difference across the capillary membrane CFC was measured in the perfused hind limbs of cats and dogs by PAPPENHEIMER and SOTO RIVERA (1948) using a gravimetric method The technique developed by MELLANDER (1960) allows a similar determination using a volumetric method where normal nervous and vascular connections can be maintained With this changes in capillary filtration coefficient can be followed together with changes in pre and post-capillary vascular resistance and mean capillary pressure as well as variations in the capacitance function

One of the major factors determining the magnitude of the filtration coefficient will be the size of the capillary surface area available for filtration exchange which is dependent upon the number of capillaries actually open to flow at any given time The number of capillaries available for filtration is governed by changes in the tone of the pre-capillary sphincters Therefore changes in the tone of the pre capillary sphincters may be deduced from changes in the filtration coefficient A preliminary report of a study of sympathetic nervous control of the pre capillary sphincters in skeletal muscle was made by FOLKOW and MELLANDER (1960) and also by ROSELL and UVNAS (1960) using another technique The present investigation was concerned with extending these observations and attempting an analysis of the changes in pre capillary sphincter tone which occur when the blood supply to the region was lowered (a) by means of symphathetic constrictor fibre stimulation and (b) by partial occlusion of the arterial inflow vessel of the region and conversely when blood flow was raised by graded muscular work A relative accumulation of vasodilator metabolites must occur in the tissues both when nutritional flow is reduced

and when tissue metabolism is increased by muscular exercise. The term "vasodilator metabolites" is here meant to include all those changes in the chemical environment of the vascular smooth muscles that tend to cause vasodilatation whenever the blood flow/tissue metabolism ratio decreases. Therefore these studies were thought to give, in addition, some infomation on the competitive effects between extrinsic nervous factors and local metabolic factors in precapillary sphincter control.

Methods

a. Experimental procedures

Experiments were performed on 37 cats anesthetized with chloralose urethan (50 mg/100 mg per kg body weight). The technique used was that described by MELLANDER (1960) which should be consulted for full details. Briefly, after extirpation of the intestines the hind part of the cat was completely isolated from the upper part of the animal at the pelvic level except for the aorta, inferior vena cava and the abdominal sympathetic nerve trunks, which contain the majority of the vasoconstrictor fibres supplying the isolated hind part. The abdominal sympathetic chains were cut centrally, isolated and could be laid on electrodes and stimulated at the level of L 4—L 5 using a Grass stimulator at stimuli of 3—5 volts and 1 msec duration and at rates from 1 up to 10 imp/sec. Arterial inflow pressure to the limbs was measured by a mercury manometer inserted in the inferior mesenteric artery. The hind part was enclosed in a water filled, temperature regulated plethysmograph so that shifts in tissue volume could be recorded continuously and quantitatively. To ensure collection of blood principally from the muscle vascular bed, the tail and hind paws were excluded from the circulation by tight ligatures at the proximal end of the tail and at the ankles. Blood flow measured using a modified Gaddum recorder inserted in the inferior caval vein which was the only outflow channel from the hind limb preparation. The height at which the Gaddum recorder was mounted above the animal's heart level determined the venous outflow pressure level and this level could be altered by raising or lowering the recorder. The arterial inflow pressure to the hind parts could be varied by applying a screw clamp around the aorta proximally to the site of pressure recording in the inferior mesenteric artery. In this preparation the parameters measurable are 1) arterial inflow pressure, 2) venous outflow pressure, 3) blood flow and 4) tissue volume of the parts enclosed in the plethysmograph, and changes in this volume reflect changes in blood volume and in transcapillary filtration exchange, which phenomena can be separated from each other.

A somewhat similar technique was used for studying CFC in the isolated calf muscles of the cat. In this preparation the paw was removed below the ankle joint and the saphenous veins were ligated to permit recording of blood flow from a relatively pure muscle region. The preparation was fixed in a water filled plethysmograph by a clamp applied to the ankle. The thigh muscles were divided just above the knee joint and the femur drilled open and plugged to remove any vascular connections. The muscles were then left supplied only by the cognate artery and vein and the nerve supply. The muscle volume was recorded from the plethysmograph, blood flow from the cannulated popliteal vein using an ordinate drop recorder (which was better suited for measuring these smaller blood flows) and pressure from the femoral artery of the opposite hind limb. Venous outflow pressure could be adjusted by changing the level of the drop recorder.

Graded muscular work was induced by direct somatomotor nerve stimulation at rates of 0 5—8 imp/sec and at 0 5—4 volts 0 1 msec. CFC was measured in this preparation at resting control levels and at different levels of muscular activity

b Method for determination of CFC

In order to measure the CFC the venous outflow recorder was first adjusted to a height such that no net transcapillary exchange of fluid occurred and the tissue region under study remained at a steady isovolumetric level The outflow recorder was then raised or lowered each time by a standard amount equivalent to the application of a venous outflow pressure of 5 mm Hg When the venous pressure was raised the volume increased due to distension of the capacitance vessels and thus first phase was followed by a second phase of slower but continuous volume increase indicating a slow accumulation of fluid within the enclosed tissues (Fig 1) due to outward filtration from the capillaries (MELLANDER 1960) These effects were reversed on lowering the outflow pressure The second slow phase of volume increase consequent upon raising venous outflow pressure was measured in ml/min/unit tissue weight

To be able to estimate to what extent mean capillary hydrostatic pressure was affected by an increment of 5 mm Hg *of* the venous outflow pressure at least the approximate ratio of pre postcapillary resistance must be known As in these preparations the muscle vessels exhibit a considerable extent of autoregulation implying marked compensatory adjustments of the pre capillary resistance vessels whenever arterial pressure is changed (FOLKOW and ÖBERG 1961) the method of PAPPENHEIMER and SOTO RIVERA (1948) for estimation of the pre-capillary to post-capillary resistance ratio cannot be used Therefore it was assumed in this series of experiments that mean hydrostatic capillary pressure was 20 mm Hg at the isovolumetric state as blood colloid osmotic pressure tissue pressure etc could be considered to be within the normal range Actually should mean capillary pressure be closer to 25—30 mm Hg or 15 mm Hg the error in deductions of the CFC would be fairly small and of about the same magnitude in all experiments where arterial pressure was in the normal range If the ratio is taken to be 4/1 at an arterial pressure around 100 mm Hg and a venous outflow pressure close to zero it means that 4 5 of a venous pressure increment of 5 mm Hg 4 mm Hg is transmitted to the capillary level (see PAPPENHEIMER and SOTO RIVERA 1948) Should in reality mean capillary pressure be 30 mm Hg instead of 20 mm Hg as here assumed 7/10 of 5 mm Hg 3 5 mm Hg would be transmitted to the capillary level The error of calculation based on the assumption of a given mean capillary pressure is therefore quite small in the given example only some 1 —13 Errors of this magnitude are of little relevance in the present estimates of changes in CFC which are often of the order of several hundred per cent (see below) The weight of the tissues enclosed in the plethysmograph — the bones being excluded — was determined at the conclusion of the experiment All data were then available for determining CFC Such determinations were carried out first at rest when the blood flow to the hind part was lowered by stimulation of the sympathetic fibres and then when blood flow was increased by muscular work.

The situation is somewhat different in the experiments where blood flow was lowered by partial aortic occlusion Since in these experiments mean hydrostatic capillary pressure was found to stay about the same when arterial pressure was lowered from 100 to say 50 mm Hg as indicated by a maintained isovolumetric state (see below) this implied *that the ratio of pre-capillary to post-capillary resistance* was decreased from some 4/1 to about 1 5 1 by a compensatory relaxation mainly of the pre capillary vessels Hence an increment of 5 mm Hg of venous outflow pressure would at the higher pressure raise mean capillary pressure by about 4 mm Hg while at the lower pressure only some 3 mm would be transmitted to the capillary level Such an approximate

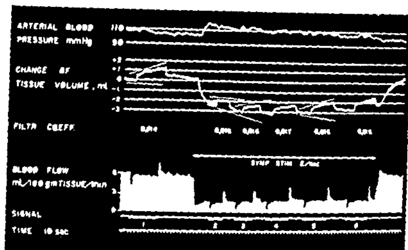


Fig 1 Effect of sympathetic stimulation on blood pressure blood flow tissue volume and filtration coefficient (measured by means of applying a standard increase of venous pressure)

correction for the applied mean capillary pressure rise was made for the calculations of CFC in the experiments where arterial pressure and hence flow was lowered by partial aortic occlusion since here such a correction is of more obvious quantitative importance

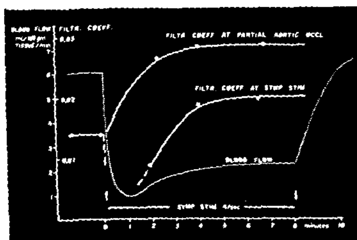
Results

1 Effect of blood flow reduction by vasoconstrictor fibre stimulation

For all resting hind limb preparations in our series values for the CFC were found to be within the range of 0 010—0 020 with a mean value of 0 015 in good agreement with the values found by the gravimetric method of PAPPENHEIMER and SOTO RIVERA (1948) and with the mean value for muscle of 0 015 given by PAPPENHEIMER RENKIN and BORRERO (1951)

Fig 1 is a record of a typical experiment in which blood flow to the hind part of the cat was lowered by stimulation of the vasoconstrictor fibres in the lumbar sympathetic trunks at 2 impulses/sec Changes in CFC were followed as rapidly as possible Before stimulation CFC was 0 014 *ml/min/100 g tissue/mm Hg* at a blood flow of 6 *ml/100 g tissue/min*

Because of the effect of sympathetic stimulation on the capacitance vessels which produced a rapid decrease in volume depending upon the decrease in local blood content (MELLANDER 1960) it was not possible to make the first measurements of the CFC until at about 30 sec after commencement of stimulation but at this time the value of the coefficient had fallen to 0 010 In fact as the ratio of pre to post capillary resistance is increased during the stimulation (MELLANDER 1960) the fraction of the increment of venous pressure transmitted to the capillary level is now slightly more than 4/5 as assumed for the resting state This means that the CFC if anything is slightly less than 0 010



Fg 2 Comp ison f ff ts on th f ltrat on coefficient of sympathet c stimulat and partial rt occlus n b th manoevres p od cing the same flow estr ct on

One of the effects of sympathetic vasoconstrictor activity appears to be to cause a constriction of the pre capillary sphincters also and the decrease in CFC reflects this effect It seems likely that a decrease of CFC to even lower values actually occurs during the initial period of vasoconstrictor activity However the initial decrease in CFC is soon followed by a gradual increase which is completed within a period of 3 min and which implies a gradual relaxation of the sphincters The coefficient ultimately rises even above the resting value implying that if anything a greater capillary surface area is now available for exchange despite maintained continued vasoconstrictor activity and lowered blood flow In the experiment illustrated in Fig 1 the coefficient rose to 0 016—0 017 at 3 min after beginning of stimulation In experiments where sympathetic stimulation more drastically reduced the flow to the muscles the CFC was sometimes even doubled after an initial decrease The secondary relaxation of the pre capillary sphincters during a prolonged constrictor fibre stimulation with a consequent reduction of flow is probably due to the action of locally accumulated vasodilator metabolites which tend to counteract the constricting influence of the sympathetic nerve stimulation The resistance vessels maintain their constriction to a considerable extent but that some secondary relaxation does occur closely correlated in time to the secondary CFC increase is indicated by the slow flow increase despite continued stimulation It should be stressed that the sphincter section forms a part of the resistance vessels apart from being a functionally specialized vascular section in its own right The capacitance vessels do not show any such relaxation on stimulation the volume fell by 3 ml and remained at this level until on cessation of stimulation it returned to the control value

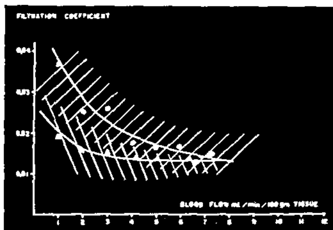


Fig 3 Cumulated data on filtrat on coeff c ents at flow restrict o ind ced by sympath t c st m lation (trian les) and partial aor t c occlus o (circles) The symbols indicat mean of classed values, th hatched areas represent approx mat range

2 Effect of blood flow reduction by partial aortic occlusion

In order to ascertain the contribution of vasodilator metabolites alone to the response of the pre capillary sphincters the blood flow to the hind part was reduced to various levels by partial occlusion of the aorta and changes in CFC were followed repeatedly and as rapidly as possible Fig 2 shows the course of CFC in a typical experiment where in the first sequence flow was reduced by a continous constrictor fibre stimulation at 4 imp/sec during which period CFC was measured In the second sequence the blood flow was reduced to the same level by means of partial aortic occlusion which caused a compensatory relaxation not only of the pre capillary resistance vessels as judged by the maintained isovolumetric state but also of the sphincter section to judge from the increase of the CFC from the resting value of 0 014 to 0 028 Comparison shows that for an equivalent blood flow the CFC is always lower when the constrictor fibres are activated This finding is also illustrated in fig 3 where the CFC determined in all experiments of this type are plotted against blood flows the values for both being at the steady state level i e performed after the secondary changes of tone of the sphincter smooth muscles occurrin during the first few minutes have been completed The triangles refer to sympathetic stimulation experiments and the open circles to mechanical flow reduction experiments These symbols each indicate classed mean values of a total of about 150 determinations in 20 cats and the range is shown by the oblique lines In general the lower the blood flow the greater is the CFC other things being equal and therefore the more pronounced the relaxation of the pre capillary sphincters presumably caused by the local chemical changes during the relative ischaemia On the other hand on drastic lowering of the arterial pressure the transmural pressure within the pre capillary sphincter section may become so reduced that a critical closing occurs even if the mooth muscle cells are quite relaxed In such a situation CFC can

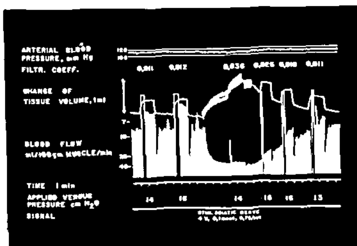


Fig 4 Effect of exercise on blood pressure muscle blood flow tissue volume and filtration coefficient.

be expected to decrease again which was occasionally observed when blood pressure was very much reduced The competition between the effect of vaso dilator metabolites accumulating at low blood flows and the constrictor fibre influence on the sphincters is apparent at each level of flow from the consistently lower values for CFC during stimulation of the vasoconstrictor nerves

3 Effect of graded muscular work

In experiments on the pure muscle preparation of the calf it was found that CFC was 0 015 (0 010—0 020) during resting steady state condition Graded muscular work at successively greater intensities induced successively greater blood flows Together with this exercise hyperemia there was a con siderable increase in CFC (see Fig 4) After the work period the CFC gradually returned to normal along with the recovery of tone of the resistance vessels — Fig 5 demonstrates classed mean values and the range of the CFC at various flow levels induced by graded muscular work. These data are based on 160 observations in 12 cats At high intensities of work maximum blood flow values were of the order of 40—50 ml blood/min/100 g of muscle and CFC reached values as high as 0 030—0 050 This represents about a threefold increase in filtration coefficient above the resting level and — if it is assumed that the capillaries contain approximately the same number and size of pores which is by no means certain — roughly a threefold increase of the size of the capillary surface area that is open to blood flow (see below) Fig 6 summarizes the results of the three procedures described above and shows the capillary filtration coefficient in the ranges of normal blood flow during mechanical and nervous flow restriction and during flow increase due to muscular work.

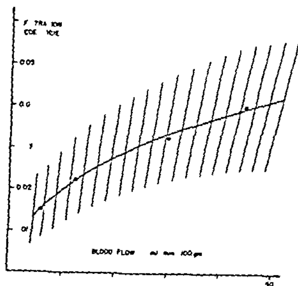


Fig 5 Cumulated data on the change of the filtration coefficient at increased blood flows, induced by means of graded exercise Dots indicate means of classed values hatched area represents approximate range

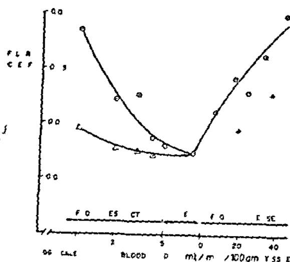


Fig 6 Cumulated data on the change of the filtration coefficient at flow restriction due to sympathetic stimulation and partial aortic occlusion and at flow increase due to muscular work. Not log scale

Discussion

The value of the coefficient for capillary filtration which is used here as an indicator of changes in pre capillary sphincter tone varies for different tissues (RENKIN and PAPPENHEIMER 1957) In the present experiments the CFC has been determined by a volumetric method for the cat hind limb preparation which includes both skin and muscle but with a great predominance of the latter (almost 95 %) since the large skin circulation of the paws and the tail was excluded The values obtained at rest with this technique correspond closely with those found by other methods and were in the range 0 010—0 070 with a mean value of 0 015

The filtration coefficient for any given tissue may be expected to be determined by the size of the capillary surface area available for filtration exchange and by the density and dimensions of its pores There seems to be no reason why the procedures carried out in our experiments should alter the permeability properties of the capillary wall (cf KORNER 1959) It could however be considered that an increase in mean capillary pressure as during muscular work might mechanically distend the capillaries passively and thereby alter the permeability of the capillary wall This possibility is highly unlikely for the following reasons During maximal work hyperaemia mean capillary pressure only exceptionally appears to rise more than some 10 mm Hg (KJELLMER 196[illegible]) Similar increases of mean capillary pressure caused by raising the venous outflow pressure in the resting equilibrium do not increase CFC which proves that transmural pressure rises of this order of magnitude do not widen the capillary pores significantly Moreover since CFC was considerably increased even when mean capillary pressure was not raised as during regional flow reduction or when muscular work as performed at a lowered arterial pressure so as to maintain an isovolumetric state such a mechanical factor seems to be of negligible importance for the changes in CFC here observed It is on the other hand well known from direct microscopic observations of the skeletal muscles that additional capillaries open up during muscular work Accordingly it appears reasonable to assume that the observed range of changes in CFC is caused by changes in the size of the capillary surface area available for the blood flow CFC would therefore also reflect changes in pre capillary sphincter tone since these sphincters control the number of capillaries that are open to blood flow In view of the satisfactory agreement with other methods the method of MELLANDER (1960) used here seems well suited to the study of sphincter behaviour

The fact that the pre capillary sphincters are responsive to vasomotor nerve stimulation was suggested by a study of BUCHERL and SCHWAB (1952) and reported also by FOLKOW and MELLANDER (1960) ROSELL and UVNÄS (1960) and RENKIN and ROSELL (1962) and the present findings confirm this The capillary surface area governing exchange with the tissues is exposed to constrictor fibre control by way of the pre capillary sphincters and although it was generally not possible to measure the filtration coefficient in the earliest stages of sympathetic stimulation the first values obtained show that within the first minutes of vasoconstrictor activity the capillary exchange surface area can be considerably reduced indicating closure of many pre-capillary sphincters As well as affecting the sphincters sympathetic stimulation caused a marked decrease in blood flow due to constriction of the resistance vessels mainly the pre-capillary ones Over the initial period of up to 3 min the closure of the sphincters is associated with a fall in mean capillary pressure caused by the increased pre to post-capillary resistance ratio and there is a tendency for inward filtration if the pressure drop is not compensated for by a corresponding

increase in the venous pressure That this tendency for inward filtration on sympathetic stimulation generally lasts for only the first minutes of stimulation under the present experimental circumstances was shown in the experiments of MELLANDER (1960) where the slope of the volume curve decreased at first but then tended to level off Although part of this decrease could be due to the fact that inward filtration at low flows might be selflimiting due to e g a decrease of the protein osmotic pressure gradient across the capillary wall it probably represents also to a great extent the beginning of relaxation of pre-capillary sphincters and adjacent sections of the pre-capillary resistance vessels and on continued stimulation this relaxation becomes more pronounced as indicated by the increasing CFC

It is perhaps not unexpected that a situation with reduced nutritional blood flow as arises with activation of the vasoconstrictor nerves should be compensated for by some mechanism which relaxes the sphincters as indeed does occur within a few minutes This would have the consequence that the reduced flow was spread over a greater capillary network governing the exchange since a better contact between blood and tissue is then established Presumably the neurogenic increase in sphincter tone is easily overcome by the influence of locally produced vasodilator metabolites which will inevitably accumulate during the period in which nutritional flow to the tissues is restricted by the constrictor fibres If however flow is artificially kept constant during a constrictor fibre stimulation the closing of the pre capillary sphincters is more easily revealed (RENKIN and ROSELL 1962) It should however be pointed out that normally a constrictor fibre activation is practically always combined with a decrease of blood flow and the present experiments then show that in the inevitable competitive interaction between the constrictor fibre transmitter and locally accumulated vasodilator metabolites the latter factor tends to dominate as regards the tone of the pre-capillary sphincters This vascular section can be said to subserve mainly the local needs of the tissues rather than being an important mechanism for the central control of cardiovascular homeostasis Accordingly for all practical purpose the local tissue control gains a considerable measure of ascendancy over the nervous control in the competition at this key point in the vascular circuit which determines how many capillaries the tissue has available for exchange The resistance vessels subserving both local needs and the centrally controlled homeostasis are generally exposed to a more dominating extrinsic control than are the pre capillary sphincters and for the capacitance vessels mainly subserving the centrally controlled homeostasis this extrinsic nervous dominance is still more striking (LEWIS and MELLANDER 1962 MELLANDER and LEWIS 1963) It also appears that the longer the constrictor fibre activation is continued and flow reduction persists the more effective the local dilator factors become in resisting the neurogenic effect on the sphincters Similar findings have been made by CELANDER and MÅRILD (1962) who in studies on the newborn child

observed a tendency for a progressive increase in CFC during prolonged reflex vasoconstrictions

Just as the vasodilator metabolite concentration of a tissue will increase with decrease of the blood flow so also will there be an increased concentration of such factors when metabolism is accelerated The performance of muscular work will readily lead to a large production of metabolites whose dilating influence is reflected by the large increase in flow and in CFC There is a correlation between the intensity of work the blood flow and CFC (Fig 5) and at maximal flows the CFC increases by some 3 times above the resting value *i e* changing from the mean resting value of 0 015 to 0 040—0 050 This increase reflects the increased capillary supply of exercising muscle (cf KROGH 1929) although the increase in the number of open capillaries reported by KROGH was greater than would be assumed on the basis of the present estimation of CFC increase Possibly this quantitative difference is due either to a smaller surface area per capillary unit of those opened up during work or to the fact that they have fewer — or smaller — pores In a few observations of potassium transfer in skeletal muscle during rest and work RENKIN (1959) also noted no more than a threefold increase of what he called the permeability surface area product

The key factor governing the direction and magnitude of capillary filtration exchange is the mean capillary pressure (PAPPENHEIMER *et al* 1948) and in our experiments the mean capillary pressure tended to stay roughly the same when arterial pressure was lowered due essentially to the compensatory relaxation of the pre capillary resistance vessels (see also FOLKOW and ÖBERG 1961 STAINSBY and RENKIN 1961) During muscular work also there occurs a considerable decrease in resistance mainly on the pre capillary side Here however arterial blood pressure is maintained and then mean capillary pressure must rise considerably This may amount to about 10 mm Hg and consequently entails a very rapid outward filtration from the capillaries at least initially the more so as now the entire capillary surface area is available (KJELLMER 1962) This is in accordance with previous findings of fluid loss from the circulation to exercising muscle (RANKE 1865) For such reasons the method of calculation used here to estimate filtration coefficients during muscular work [illegible] assumes that the ratio of pre to post capillary resistance is 4 to 1 [illegible] underestimates the filtration coefficient If mean capillary pressure [illegible] intense muscular work increases 10 mm Hg at an arterial pressure of [illegible] ratio is closer to 7 to 3 Hence the maximal CFC values given [illegible] be some 15 per cent lower than the true values

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From the Department of Physiology Veterinarhogskolan Stockholm 51 S eden

Effect of Salt on Sugar Response

By

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Rece ed 2? July 196?

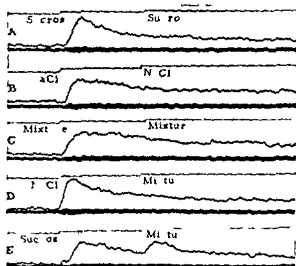
Abstract

Funakoshi M and Y Zotterman *Effect of salt on suga response* Acta physiol cand 1963 57 193—200 The integrated responses to 0 5 M suc ose 0 5 M sodium chloride and a mixed solution con taining 0 5 M sucrose and 0 5 M odium chloride were recorded f om the whole chorda tympani of the dog The mixed olut on was applied on the dog s tongu after application of sucrose o sodium chloride ith out water rinsing b tween them Thus it was found that 0 5 M sodium chloride depressed the response to the m xture applied after the salt sol tion h l the esponse to m xture as n ver depressed by previous 0 5 M ucrose Single fibr analys s sho ed that the response to 0 5 M sucros of single sugar fib es was dep essed entir ly by previous 0 5 M sodium chlo ide while the response to 0 5 M sod um hlo ide of s ngle salt fib es was never affected by a pre ous appl cation of 0 5 M sucrose and that the response of ngle suga salt fib es to the mixture was depressed partially by od um chlorid but not by ucrose

Many nvestigations on taste have appeared in the literature however there have been few studies on masking or inhibition with taste mixtures Many beverages are based on the principle that sugar reduces the acid taste Andersson *et al* (1950) sho ed that the mixture of acid pH 2 5 and 10 sucrose gave an algebraic sum of the responses to acid and to sucrose as far as impulse frequency was concerned Thus they suggested that there were specific endings for sweet and acid solution and the depressing effect of sugar on the acid taste must has its seat centrally

In the present paper the peripheral depressing effect of salt on the response to sugar has been studied

On l fr m Dep tment of Phys logy Dental School Osaka [illegible]



F g 1 Integrated responses reco ded from w hol cho da tympani nerve upon appl cation fi e d fferent combinations of 0 5 M su rose a d 0 5 M sod um chlo de Note the depres ng eff ct of sod m chloride on the response to the mixt re Tim in ec

Method

Seventeen mongrel dogs (male and female their body weights 3 5—11 kg) were used in the present experiment Under Mebumalnatrium anesthesia (0 6 ml/kg body w eight on ml of w hich contains 18 mg mebumal 40 mg mebumalsod um and 250 mg urethane the chorda tympani was exposed The integrated response of the w hole chorda tympani and s ngle spike discharge of a fine strand were recorded The operative procedure and exp rimental technique w ere almost the same as those described in previous papers from this laboratory (ZOTTERMAN 1936 ANDERSSON et al 1950 LILJESTRAND and ZOTTERMAN 1954 COHEN HAGIWARA and ZOTTERMAN 1955) As taste solution 0 5 M suc ose 0 5 M sodium chloride and mixed solution conta ning 0 5 M sucrose and 0 5 M sodium chloride were used These solutions w ere kept at 38 C and applied to the dog s tongue by means of the t o-cyl nder appl er w hich was dev ced to give tw o d ffe ent solut ons successively one after another w ithout water rinsing bet een them Tl e volume of each test solut on applied to the tongue was about 5 ml The mixed solut on w as applied after appl cation of 0 5 M sucrose or 0 5 M sodium chloride

Results

Integrated responses

The integrated responses of the whole chorda tympani were recorded in seven dogs The integrated responses to 0 5 M sucrose 0 5 M sodium chloride and a mixed solution containing of 0 5 M sucrose and 0 5 sodium chloride were similar in most of our chorda tympani preparations

In Fig 1 records A B a d C show the adaptation to each taste olution when the same solution was succe sively applied to the tongue without water rinsing i e 0 5 M sucrose after 0 5 M sucrose 0 5 M sodium chloride after 0.5 M sodium chloride and 0 5 M mixture after 0 5 M mixture In every case there

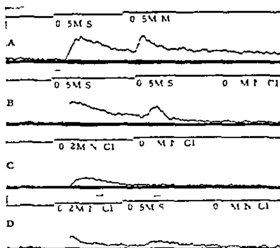


Fig 2 D p s ing ff ct of 0.2 M sod um hloride on th espon e to mixture onta ning 0 5 M ucros and 0 2 M sod um chlo id T m 1 sec.

was no response to a second application of the identical solution Then the mixture was applied to the tongue after the application of 0 5 M sucrose and after 0 5 M sodium chloride As shown in Fig 1 D and E the mixture did not induce any response when applied after 0 5 M sodium chloride while the mix ture produced a conspicuous response following upon 0 5 M sucrose

Even when the strength of the sodium chloride of the mixture was lowered to 0 2 M a quite strong response to this mixture appeared after a previous application of 0 5 M sucrose (Fig 2 B) After the application of 0 2 M sodium chloride there was a very small response to the sucrose of the mixture as will be seen from Fig 2 D These results indicate that sucrose did not interfere with the response to the lower concentration of sodium chloride while on the other hand the lower concentration of sodium chloride still exerted a depressing effect on the response to the sucrose

For the further analysis of these phenomena we turned to a study of the response of single gustatory fibres responding to sugar

Single fibre analysis

Altogether 32 functional single fibres including 8 sugar fibres 13 sugar salt fibres 5 salt fibres and others were analyzed

Sugar fibre

All sugar fibres which responded to 0 5 M sucrose but neither to 0 5 M sodium chloride nor to water were inhibited by previous 0 5 M sodium chloride

Fig 3 shows the typical response of the sugar fibre This fibre did not respond to water but it was stimulated by 0 5 M sucrose (Fig 3 A) As shown

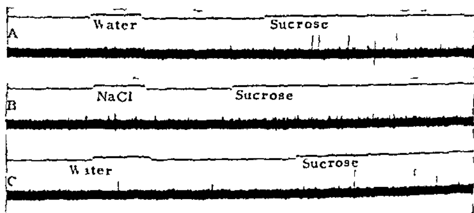


Fig 3 Records from a single sugar fibre Response to 0 5 M sucrose is inhibited by previous 0 5 M sodium chloride Time in sec

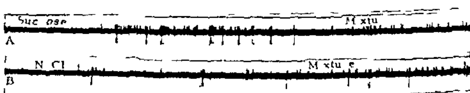


Fig 4 Responses recorded from a single sugar salt fibre (type I) upon application of 0 5 M mixture after 0 5 M sucrose (A) and 0 5 M sodium chloride (B) Time in sec

in Fig 3 B the application of 0 5 M sucrose following previous 0 5 M sodium chloride did not stimulate the fibre Fig 3 C presents the reproducibility of the response to 0 5 M sucrose after water For four of eight sugar fibres the response to sucrose after a previous sodium chloride application was totally suppressed In the other four fibres it was also almost entirely abolished

Sugar salt fibre

Sugar salt fibres were stimulated by 0 5 M sucrose as well as by 0 5 M sodium chloride but their response to these stimuli were not uniform Hence they have been classified tentatively into three subdivision i e type I type II and type III Type I is the sugar salt fibre which responds much stronger to 0 5 M sucrose than to 0 5 M sodium chloride Type II is the salt sugar fibre which responds to 0 5 M sodium chloride much more than to 0 5 M sucrose And type III is the intermediate type of fibre which is almost equally sensitive to both stimuli

The response of type I sugar salt fibre to 0 5 M sucrose 0 5 M sodium chloride and a mixed solution of them are reproduced in Fig 4 The activity in this fibre resulting from stimulation with 0 5 M sucrose was 14 spikes per second (Fig 4 A) and that of 0 5 M sodium chloride was 4 spikes per second (Fig 4 B) The discharge frequency of this fibre to the sugar salt mixture

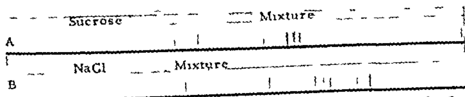


Fig 5 Responses recorded from a single salt sugar fibre (type II) upon application of 0 5 M mixture after 0 5 M sucrose (A) and 0 5 M sodium chloride (B) Time in sec

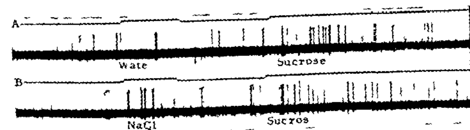


Fig 6 Responses recorded from a single fibre of type III Note the response to 0 5 M sucrose is depressed by previous 0 5 M sodium chloride Time in sec

applied after the response to 0 5 M sucrose was 6 spikes per second and the response to the mixture after 0 5 M sodium chloride was also 6 spikes per second Other fibres of type I showed almost the same activity to the mixture after 0 5 M sucrose or 0 5 M sodium chloride

Fig 5 shows the response of one of type II salt sugar fibre The response of this fibre to 0 5 M sucrose was 2 spikes in the first second (Fig 5 A) and that to 0 5 M sodium chloride was 7 spikes (Fig 5 B) In this fibre the mixture produced 6 spikes in the first second after the response to 0 5 M sucrose and 4 spikes after 0 5 M sodium chloride Most of the other fibres of this type showed a similar behaviour

The depressing effect of salt on the sugar response was observed more clearly in the type III intermediate fibres than in type I and type II fibres As shown in Fig 6 one of type III fibres responded to 0 5 M sucrose significantly with discharge at 11 spikes per second (Fig 6 A) and also responded to 0 5 M sodium chloride with 12 spikes (Fig 6 B) However after the response to 0 5 M sodium chloride the activity of this fibre to 0 5 M sucrose was depressed to 6 spikes per second (Fig 6 B) A similar effect of salt on the sugar response was illustrated in Fig 7 The electrical activity to 0 5 M mixture in this preparation was not inhibited by previous 0 5 M sucrose (Fig 7 A) but depressed by 0 5 M sodium chloride (Fig 7 B)

Salt fibre

All the salt fibres which responded to 0 5 M sodium chloride but not to 0 5 M sucrose or water were never affected by 0 5 M sucrose applied to th

The demonstration of LOEWENSTEIN (1958) that the specific structure of the pacinian corpuscle hardly has anything to do with the transduction of the stimulus as all the leaves of the onion can be pealed off without interfering with the excitatory process shows that the specific property of the receptor is inherent in the ending of the naked nerve fibre and not in the corpuscle Thus there is every reason to accept the possibility that fine nerve endings pene trating the mucous membrane could serve as specific gustatory receptors Further we might have both mechanisms which each served different purposes *i e* villi and gustatory epithelial cells as receptors as well as specific free gustatory nerve endings

The results obtained in this and previous studies on single gustatory nerve fibres point in the direction that most gustatory nerve fibres respond to more than one class of sapid substances while a few fibres are more or less specifically responding only to one quality of stimulus and not to others within reasonable stimulus strength The experimental data so far do not give us any clue to determine whether the gustatory cell including its villi is specific or not If that was so the multi quality response should then depend upon the fact that dif ferent kinds of gustatory cells are supplied by the same nerve fibre Whichever is the fact the present study shows that sodium chloride exerts a strong depressing effect upon the gustatory structure sensitive to sugar whether this structure is inherent in the villi of gustatory cells or in free endings of gustatory nerve fibres

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Elimination of Ethanol as a Measure of the Hepatic Blood Flow in the Cat

I. Experiments with single injection of ethanol

By

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Abstract

LARSEN, J. A. *Elimination of ethanol as a measure of the hepatic blood flow in the cat. I. Experiments with single injection of ethanol.* Acta physiol. scand. 1963. 57. 201—208. — The elimination curve following a single injection of 0.25 g ethanol per kg weight was studied in 13 cats anesthetized with Nembutal. It was found that the elimination was constant down to arterial concentrations of 40—60 mg/l, the mean value being 103.1 mg/kg/h. Below these concentrations the extraction in the liver was almost complete and the amount metabolized in the liver was thus dependent on concentration and liver blood flow. After correction for the amount of ethanol eliminated outside the liver the hepatic blood flow was calculated from the elimination curve. A mean value of 34.3 ml/kg/min was found.

It has been widely accepted that the initial oxidation of ethanol takes place exclusively in the liver and consequently ethanol should be suited for determination of the liver blood flow using the Fick principle. In initial experiments on humans (LARSEN 1959) it was found that below certain small concentrations ethanol was almost completely extracted in the liver. Under these circumstances the arterio-hepatic venous difference can therefore be determined without liver vein catheterization. Two methods for determination of the liver blood flow without liver vein catheterization were suggested, one using a single injection, the other using continuous infusion of ethanol.

However, the results obtained by the infusion method on human subjects indicated the existence of a considerable extrahepatic elimination of ethanol. The amount could be determined indirectly and a mean value of 20.5 mg/min

was found (LARSEN 1959 a b) Later experiments with determination of the liver blood flow by means of bromsulphalein partly confirmed these results An extrahepatic elimination was found averaging 15 3 and 12 5 mg/min (LARSEN TYGSTRUP and WINKLER 1961)

For further confirmation of the results obtained by these methods a series of experiments were carried out on cats In the experiments reported in this paper the elimination curve after a single injection of ethanol has been studied with calculation of the elimination rate and the extraction of ethanol in the liver Finally the elimination curve has been used for determination of the liver blood flow

Experimental Procedures

The condition of the animals The cats were fasted for 15 hours but were permitted water ad libitum They were anesthetized using 3 5 per cent solution of ethyl methyl butyl barbituric acid (Nembutal) given intraperitoneally (35 mg/kg body weight) and were maintained at a surgical level by repeated intravascular injections The blood pressure was recorded during the whole procedure and the temperature controlled and kept constant within one degree (C) Cats are very sensitive to intravascular injections of Nembutal and therefore the sustaining doses must be given in small amounts and very slowly If not a long lasting fall in blood pressure is noted The blood pressure should be as constant as possible in order to avoid alterations in the liver blood flow The amount of Nembutal necessary for maintaining anesthesia varied greatly from cat to cat but was usually about 18—36 mg/h The depth of anesthesia could be followed by observing the degree of muscle tonus the pupils and various reflexes as well as the blood pressure which tended to rise as the anesthesia wore off

Collection of blood samples Arterial samples were used drawn from a catheter placed in the femoral artery This catheter was also used for the initial injection of ethanol and the sustaining doses of Nembutal In some experiments catheterization of a liver vein was performed The catheter was introduced through the right external jugular vein and placed deep in a liver vein under fluoroscopic control

Determination of ethanol The ethanol content was determined on plasma by means of an enzymatic method using alcoholdehydrogenase (ADH) (BUCHER and REDETZKI 1951 LUNDQUIST and WOLTHERS 1958 LARSEN 1959 a) Two buffer solutions were used. Buffer I consists of Sodium pyrophosphate A R (10 g) semicarbazide HCL (2 5 g) glycine Sørensen (0 5 g) 2 N NaOH (10 ml) and water to make a final volume of 300 ml pH 8 6-8 8 Buffer 2 consists of Glycine Sørensen (15 g) ethylenediamine tetra acetate (0 3 g) semicarbazide HCl (2 5 g) 2 N NaOH (25 ml) and water to make a final volume of 200 ml pH 8 8—8 9

0 5 ml of plasma was deproteinized with equal amounts of perchloric acid 5 1 per cent (w/v) and 0 8 ml of the supernatant was neutralized with 0 04 ml 4 N NaOH For concentrations between 300 and 20 mg/l the following procedure was used Buffer I ADH (Boehringer 30 mg/ml) and diphosphopyridinnucleotide (DPN Boehringer reinst 15 mg/ml) were mixed in the proportion 300/10/1 and distributed in 6 ml test tubes in 3 ml portions 0 1 ml of the neutralized supernatant was added the sample was gently shaken and then allowed to stand undisturbed for 60 minutes The amount of reduced DPN was then measured in a Beckman spectrophotometer (DU) at 340 nm A water blind and two ethanol standards were treated in exactly the same way as the plasma and from the standards a factor was determined by which the extinction value was converted to ethanol concentration In this way the ethanol content in plasma was

Table I The results from a single injection of 0.25 g ethanol/kg body weight (— indicates experimental failure)

Cat n	Weight (kg)	α value	Elimination of ethanol in different terms				Conc at the flattening of the elimination curve mg/l plasma	Hepatic plasma flow corrected for			
								Directly determined extrahepatic elimination of ethanol		Indirectly determined extrahepatic elimination of ethanol	
			mg/kg/h	mg/100 g liver/h	mg/l plasma/h	mg/min		ml/min	ml/kg/min	ml/min	ml/kg/min
1	4.3	0.70	89.6	—	118	5.92	50	—	—	102	23.7
2	2.1	0.72	116.6	—	15	3.81	40	78	37.1	68	32.4
3	2.4	0.62	100.4	241.2	162	4.02	50	66	27.5	—	—
4	4.9	0.68	91.8	330.0	135	7.42	50	124	25.3	—	—
5	2.6	0.67	93.2	255.6	148	4.35	40	94	36.2	86	33.1
6	4.8	0.59	89.7	409.8	152	7.17	50	123	25.6	127	6.5
7	3.6	0.66	108.2	388.8	164	6.48	60	91	25.3	88	24.4
8	3.5	0.76	91.2	318.0	120	5.30	40	112	32.0	93	28.0
9	4.8	0.58	104.4	300.0	180	8.35	50	141	29.4	—	—
10	4.1	0.63	104.0	266.4	165	7.02	60	104	25.4	97	23.7
11	4.5	0.58	140.9	447.6	243	10.45	50	187	41.6	193	42.9
12	4.8	0.59	121.5	373.8	206	9.72	60	147	30.6	160	33.3
13	5.3	0.68	95.2	288.3	140	8.41	—	—	—	—	—
	Mean	0.65	103.1	329.0	161	6.80	50	115	30.6	113	29.8
	S.D.	0.06	15.3	67.8	34	2.11	7.4	35	5.6	40	6.3
	Coefficient of variation	9.7	14.8	20.6	21.4	31.0	14.8	29.9	19.8	35.4	21.1
							Hepatic blood flow	132	35.2	130	34.3

determined but if wanted the concentration in whole blood can be calculated by multiplication with (100—0.4 H)/100 where H indicates the haematocrit value.

For concentrations below 20 mg/l the following modification was used. Buffer 2 and DPN were mixed in the proportion 20/1 and to one half of the mixture ADH was added the amount being 1/20 of the total amount of DPN used. 0.3 ml neutralized supernatant was then added to 0.6 ml of each buffer mixture and the extinction value read at 340 nm after 60 min. The value of the sample without ADH was subtracted from the value of the sample containing ADH and the difference used for calculation of the ethanol content in the manner described above. This is necessary because the supernatant in this modification has a significant absorption at 340 nm.

These procedures have a very high degree of specificity and accuracy. The modification used for values between 300 and 20 mg/l has a coefficient of variation of 0.7 per cent and the recovery is 100.2 per cent. The modification used for values below 20 mg/l has a coefficient of variation of 2.2 per cent and the recovery is 100.0 per cent.

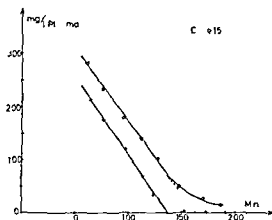


Fig 1 The elimination curve from a single injection of 1 13 g ethanol O—O = Femoral artery Y—X = Hepatic vein

Determination of the hepatic blood flow 0 25 g ethanol/kg body weight was given as an 8 per cent (w/v) solution in isotonic sodium chloride through the catheter in the femoral artery and the elimination curve was determined from arterial blood samples taken at intervals of 15—20 min for 3 hours When distribution is complete (after 45—60 min) the elimination curve is rectilinear signifying a constant elimination of ethanol. Corresponding to the rectilinear part of the curve the liver is supplied with more ethanol than can be oxidized and the metabolic capacity of the liver is thus the rate limiting factor From the elimination curve the total amount of ethanol metabolized per minute can be calculated as shown by WIDMARK (1932 p 43)

At a concentration of 40—60 mg/l plasma the elimination curve begins to flatten and gradually approaches the abscissa It has been shown by liver vein catheterization in human subjects (LARSEN 1959 a) that during the rectilinear part of the elimination curve the concentration in the liver vein runs below and parallel to the arterial concentration. When the arterial curve begins to flatten the concentration in the liver vein becomes zero From now on the amount of ethanol brought to the liver by the blood is insufficient to saturate the ethanol oxidizing system Thus the ethanol content of the blood limits the amount metabolized in the liver

Consequently the arterial concentration at which the elimination curve starts to flatten must represent the arteriohepatic venous difference in the preceding period According to the Fick principle the hepatic blood flow is calculated by dividing the amount metabolized per minute in the liver at complete saturation by the concentration in the blood at which the curve starts to flatten The amount metabolized in the liver is calculated by subtracting the amount eliminated outside the liver from the total amount eliminated (This extrahepatic elimination was determined in each cat indirectly as well as directly in experiments reported in a subsequent paper (LARSEN 1963))

It should be mentioned that in the present experiments plasma values have been used for calculation of the liver flow The value obtained is called plasma flow but it is not identical with the plasma flow obtained by using bromsulphalein and similar compounds which are bound to the plasma proteins Ethanol is distributed equally in the water phase of plasma and erythrocytes and the flow calculated from the plasma concentration of ethanol will therefore be greater than the actual plasma flow In Table I the mean plasma flow has been converted to blood flow by multiplication by 100/(100—0 4 H) where H is the mean haematocrit 33 4 ± 3 75 from 10 cats chosen at random.

Table II The elimination rate and r value (s e t xt) found by different authors The peripheral concentration at which the elimination rate was calculated is listed in the first column

Author	Peripheral concentration (mg/l)	Elimination rate		r value
		mg/kg/h	mg/100 g liver/h	
Newman and Lehman 1937	2 000	14?	—	0 74 (0 70—0 75) blood values
Clark et al 1939	600	141	—	—
Eggleton 1940	1 000—2 000 100— 200	114—150 81—120	460 280—339	0.59 (0 48—0 78) plasma values
Hei et al 195?	1,200	126	—	0 74 (0 72—0 75) blood values
Larsen 1962	100— 200	103	333	0 65 (0 58—0 6) plasma values

Results

The results from 13 experiments are shown in Table I The elimination rate of ethanol and hepatic flow is expressed in different ways to facilitate comparison with results published by other authors For the same reason the liver blood flow is given both as ml/min and ml/kg/min In the table is also listed the r value which is the part of the body in which ethanol is dissolved to the same extent as in plasma In calculating the liver blood flow the total amount of ethanol eliminated has been corrected for the amount of ethanol eliminated outside the liver determined as well directly as indirectly

An experiment with a single injection of ethanol and liver vein catheterization is illustrated in Fig 1 The arterio-hepatic venous difference is seen to increase during the experiment indicating a fall in the blood flow In some experiment it was found that although the elimination curve was flattening there were still small amounts of ethanol in the blood taken from a liver vein This does not necessarily mean that the extraction of ethanol is incomplete but might be explained by contamination with blood from the inferior vena cava or afflux of small amounts of blood shunted past the liver cells

The amount of ethanol eliminated in the expired air and excreted in the urine has not been measured as the amount lost through these ways plays an insignificant role in the elimination when only small plasma concentrations are present

The fasting value of ethanol in the plasma has been found to be very l w In 26 d terminations the plasma contained no ethanol in 11 cases The mean value was 0 4 mg l plasma with a standard deviation of 0 4? mg/l

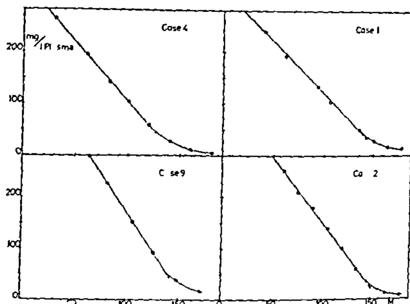


Fig 2 The elimination curves from a single injection of 0 25 g ethanol/kg body weight

Discussion

The elimination rate of ethanol In the literature only few experiments are reported in which the elimination rate of ethanol has been determined in cats The results of these experiments are listed in Table II It is seen that the elimination rate varies considerably as is the case in other species including man The results in Table I indicate that the most constant value is found i the metabolic rate is expressed in relation to body weight though it might be expected that the findings expressed in terms of liver weight would give more constant values

The elimination rate found in the present experiments is low but is in accordance with the elimination rate found by EGGLETON (1940 a b) at comparable small concentrations In earlier experiments on humans (LARSEN 1959 a) where the elimination rate was calculated at similarly small concentrations (200—100 mg/l blood) the amount eliminated was also found to be smaller than usually reported (66 mg/kg/h against 84—132 mg/kg/h (ELBEL and SCHLEYER 1956 p 48)) This brings up the question of whether the elimination of ethanol is dependent on the concentration An extensive review on this matter is given by JACOBSEN (1952) and ELBEL and SCHLEYER (1956 p 56—59)

The fact that the elimination of ethanol in the experiments published in the present article is smaller than usual points to a relationship between elimination rate and concentration Nevertheless as in the experiments on humans (LARSEN 1959 a) the elimination curves were always found to be rectilinear from the

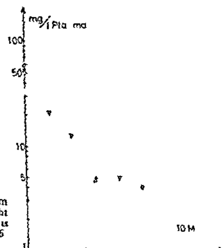


Fig 3 The last part of the elimination curves from a single injection of 0 25 g ethanol/kg body weight plotted semilogarithmically The abscissa unit is 10 Min ○—○ = case 2 ●—● = case 6 ×—× = case 7 ⌴—⌴ = case 8

point where diffusion equilibrium was established and down to concentrations about 50 mg/l plasma Some typical curves are shown in Fig 2 Mathematical analysis of the rectilinear part of the curve has not been found necessary The significance of the curve below 50 mg/l is rather obscure MARSHALL and FRITZ (1953) have explored the same concentration range and found the curves to be rectilinear in a semilogarithmic system which means that the elimination is proportional to the concentration In some cases I have found the same but in most cases the curves are rather irregular Some examples are given in Fig 3 However a rectilinear course in the semilogarithmic system can not be expected as the elimination below 50 mg/l will consist of a hepatic elimination depending on the concentration in combination with a constant extrahepatic elimination which finally becomes dependent on the concentration too (LARSEN 1959 a 1962) This fact taken together with small variations in blood flow and an increasing analytical error at very small concentrations will influence the results in such a way that an irregular time/concentration relationship might be expected

The influence of anesthesia on the elimination rate Whether Nembutal influences the metabolic rate in cats is uncertain as it has never been measured without anesthesia CLARK *et al* (1940) could not demonstrate any effect of Nembutal on the metabolic capacity of the livers from cats in in vitro experiments EGGLETON (1940 a b) found no significant difference in the elimination rate of ethanol in dogs with and without anesthesia By comparing the elimination rate in dogs with the elimination rate in cats anesthetized with Nembutal she concludes that this anesthetic has only little if any inhibitory action on the metabolic rate WHITTLESEY (1954) has determined the elimination of ethanol in dogs without anesthesia and anesthetized with Nembutal This investigation

involved 44 experiments on 4 dogs and by statistical analyses of the results it was found that Nembutal decreased the elimination rate with 4—18 per cent.

The liver blood flow. As in humans the extraction of ethanol in the liver seems to be complete below arterial concentrations of 50—60 mg/l blood. This means that also in cats the hepatic blood flow can be determined from the elimination curve provided the amount of ethanol eliminated outside the liver is known.

The results obtained by this method will be discussed in a subsequent paper where the results of experiments with continuous infusion of ethanol are reported.

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Elimination of Ethanol as a Measure of the Hepatic Blood Flow in the Cat

II The significance of the extrahepatic elimination of ethanol

By

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Received 30 March 196

Abstract

LARSEN J A El m nati n f ethanol as a measu e of the l jatic m the cat II The gn ficanc f the xt ahepat l mination of tl physiol scand 1963 57 209—223 — The metabol sm following cont nuous nfus on of small amounts of etl anol tudied n th rteen cats By changing the amount of etha per minut a least once in the same experiment an ex eliminat on of ethanol ould be demonstrated averaging m n In ac ordance with this esult an elim nation of et f und n the ame cat aft r eviscerat on averag ng al o 0 Th rty f per cent of this extrahepatic elimination t k kidneys Th extrah patı liminat on was found to be tant do n to rt nal concentrations of about 10—1 Th l er blood flo has been d termined by means nfus on of thanol without l ver e n catheter zat alu of 26 8 ml kg min was found Apparently the o hloralose d d not alt r th li er blood flow wl de ease n flow was not ed wh n ethe was used fo

A method fo determination of the hepatic blood flow by infusion of ethanol without hepatic vein catheterizati n suggested (LARSEN 1958 By use of this method in huma results were obtained h ch ndicated an extrahepatic The amount f ethanol metabolized utside the liver

was found to be constant in the concentration range examined *i e* from 20—50 mg/l blood In this article experiments on cats will be described which confirm these results

The liver blood flow was determined by continuous infusion of ethanol and in each cat the extrahepatic elimination was determined indirectly and later directly by determining the elimination rate after evisceration

In the eviscerated animal both the constancy of the extrahepatic elimination and the oxidizing capacity of the kidneys were examined

Experimental procedures

The condition of the animals The experiments were performed on cats in the post absorptive state and anesthetized with Nembutal as described previously (LARSEN 1963) It must be emphasized that the eviscerated cat is very sensitive to Nembutal and as the liver has been removed only small amounts of Nembutal are necessary for maintaining anesthesia In some experiments nitrous oxide + ether were used and in others chloralose

Collection of blood samples Arterial samples were taken from a catheter placed in the femoral artery In some experiments catheterization of a liver vein was performed by the same technique as used earlier on cats (LARSEN 1963)

Determination of ethanol Ethanol was determined enzymatically by means of alcohol dehydrogenase as described in detail previously (LARSEN 1963)

Determination of the liver blood flow and the amount of ethanol eliminated outside the liver A solution of ethanol in isotonic sodium chloride was infused intravenously by means of a motor driven syringe at a rate of 0 187 ml/min. The amount infused per minute was always smaller than that eliminated at complete saturation of the liver Once or twice during each experiment the concentration of ethanol in the solution infused was augmented in order to obtain different concentrations of ethanol in the blood A priming dose was given at the start of the infusion and when the amount infused was changed The calculation of this dose was based on the weight of the cat and the r value (the portion of the body in which ethanol is dissolved to the same extent as in plasma) The r value together with the amount of ethanol eliminated after complete saturation of the liver was determined in earlier experiments on each cat by means of a single injection of ethanol (LARSEN 1963)

45 minutes after the start of the infusion the concentration in the blood is constant indicating that the amount eliminated equals the amount infused In experiments with a single injection of ethanol it was found that when saturation of the liver was incomplete the concentration in the liver veins was almost zero (LARSEN 1963) This means that when the concentration in the blood is constant it is identical with the arterio hepatic venous difference Provided that ethanol is eliminated exclusively in the liver it should be possible to calculate the hepatic blood flow according to the Fick principle by dividing the amount infused per minute by the blood concentration at level

It was found however that the blood flow calculated in this way was dependent on the amount infused being smaller when the amount infused was increased This observation can be explained by the existence of an extrahepatic elimination of ethanol in the following way provided that ethanol is metabolized exclusively in the liver the concentration when constant must be directly proportional to the amount infused If the results are plotted on a graph with the amount infused as the abscissa and the concentration as the ordinate the points should lie on a straight line passing through the *0-point*. In Fig 1 an

experiment with three levels is illustrated and it will be seen that the points fall on a straight line but this line intersects the abscissa to the right of the origin This parallel displacement of the line can be explained by assuming an extrahepatic elimination of ethanol which is constant in the concentration range examined The amount eliminated extrahepatically can be calculated from the point of intersection If this amount is subtracted from the amount infused the blood flow is the same when calculated at different infusion rates

The amount of ethanol eliminated outside the liver (k) and the hepatic blood flow (EHBF)[1] can also be calculated from the following equations

$$c_2 \times \text{EHBF} + k = a_2$$
$$c_1 \times \text{EHBF} + k = a_1$$

where c_1 and c_2 are the constant concentrations when different amounts a_1 and a_2 are infused From these equations the two unknown factors k and EHBF can easily be determined This calculation has been used in the present article

As in earlier experiments only plasma values have been determined and the flow calculated is called the plasma flow It is however not identical with the plasma flow calculated by means of compounds bound to the plasma proteins *e g* bromsulphalein Ethanol is distributed equally in the water phase of plasma and erythrocytes and the plasma flow calculated from the ethanol content in plasma is therefore greater than the actual plasma flow The mean value from the experiments has been converted to blood flow by multiplication by the factor (100)/(100—0 4 H) where H is the mean haematocrit (33 4 ± 3 75) from 10 cats chosen at random

Evisceration Evisceration was performed as a two step procedure In the first operation the abdomen was opened through an incision parallel to and a little below the right curvature and the inferior vena cava was constricted to a diameter of 1—1 5 mm by means of a silk ligature tied around a probe of this diameter This was done in order to establish sufficient anastomoses between the inferior and superior vena caval systems The anastomoses are fully established after 10—12 days Then evisceration was performed leaving the kidneys in situ The liver was totally removed together with the part of the inferior vena cava situated between the diaphragm and the ligature During the experiment glucose was infused continuously at a rate of 100 mg/kg/h to substitute the normal output of glucose from the liver The animals tolerated the evisceration well but the blood pressure showed a tendency to fall during the experiment which usually lasted 4—5 hours After the evisceration a single injection of ethanol was given (0 1—0 2 g/kg) and the elimination curve studied In some of the experiments the kidneys were removed after two hours in order to establish whether ethanol is metabolized in these organs

Summary of the experimental procedures performed on each cat

1) Single injection of ethanol with cannulation of the femoral artery (The results of these experiments have been reported separately (LARSEN 1963))

2) Continuous infusion of ethanol with cannulation of the femoral artery and vein

3) Preparation for evisceration with constriction of the inferior vena cava

4) Evisceration and single injection of ethanol with cannulation of the femoral artery and vein

The first three procedures were performed under sterile conditions and as a rule the cats recovered very quickly They were often drinking and eating normally from the second day after the experiment The weight was constant or even increased in some cases There was an interval of at least 10 days between each experimental procedure

[1] (EHBF = Estimated Hepatic Blood Flow)

Table I The results from single injection experiments on eviscerated cats (— indicates experimental failure)

Cat no	r value eviscerated cat	Extrahepatic elimination of ethanol				Elimination in kidneys in per cent of total extrahepatic elimination (directly determined)
		Indirectly determined		Directly determined		
		mg/min	Per cent of total	mg/min	Per cent of total	
1	—	0.8	13	—	—	—
2	0.63	1.1	28	0.7	18	46
3	0.67	—	—	0.7	18	3[illegible]
4	0.62	—	—	1.2	16	40
5	0.64	0.9	21	0.6	14	26
6	0.56	0.8	11	1.2	17	57
7	0.62	1.2	18	1.0	15	21
8	0.6	1.4	27	0.8	15	—
9	0.62	—	—	1.3	15	35
10	0.68	1.2	17	0.8	11	—
11	0.69	0.8	8	1.1	11	21
12	0.66	0.2	2	0.9	9	—
13	—	1.0	12	—	—	—
Mean	0.65	0.94	15.7	0.94	14.5	34.8
S D	0.051	0.33	8.2	0.23	2.9	12.5
Coefficient of variation	7.8	35.1	52.2	24.5	20.0	35.9

Results

The r value The r value (the portion of the body in which ethanol is dissolved to the same extent as in plasma) has been calculated in order to ascertain that normal distribution of ethanol took place in the eviscerated animal The values for eviscerated cats are listed in Table I Taking the small day to day variations into consideration there is a very good agreement with the values obtained in the intact animals (LARSEN 1963 Table I)

The mean value in the two series is 0.65 (s d 0.05 n = 11) and 0.65 (s d 0.06 n = 13)

Extraction of ethanol in the liver In previous experiments on cats with single injection of ethanol it was found that below concentrations of about 50 mg/l plasma the extraction in the liver was almost complete (LARSEN 1963) This was confirmed in two of three experiments where liver vein catheterization was performed One experiment is illustrated in Fig 2 where the concentration in a liver vein is almost zero at an arterial concentration of about 85 mg/l

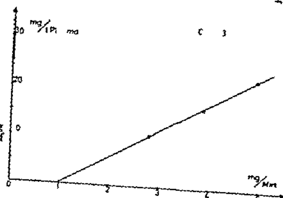


Fig 1 Mg ethanol infused per minute in relation to the corresponding arterial concentration The point of intersection with the abscissa indicates the amount of ethanol eliminated outside the liver

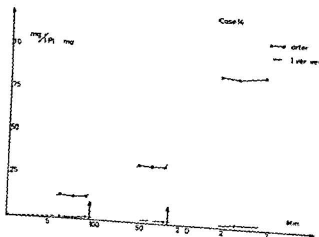


Fig 2 The concentration of ethanol in the peripheral blood and blood from liver vein after 60 minutes of constant infusion of ethanol At the arrow the amount infused per minute is augmented

plasma In the third experiment however the extraction was incomplete and different at different levels

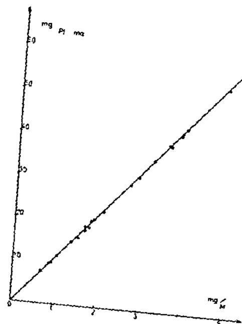
Liver blood flow and its constancy It is of great importance that the blood flow is constant during the entire experiment Thus seemed to be the case in the experiments on humans (LARSEN 1959 a b) but could not be expected a priori to be the case with anesthetized cats In two cases a constant infusion was given over a period corresponding to the length of an ordinary experiment In one case the flow seemed constant but in the other there were small fluctuations without any definite rise or fall

The constancy of the liver blood flow in the experiments can however be illustrated by plotting the results from all the experiments on the same graph but in order to make the results comparable two corrections must be made 1) The amount of ethanol eliminated outside the liver (corresponding to the point of intersection with the abscissa) must be subtracted from the amount infused in each experiment This correction should make the lines pass through

Table II *The results from continuous infusion of ethanol The arterial concentrations have been corrected by multiplication with a factor calculated by dividing the actual liver flow by the mean flow from all the experiments The amounts of ethanol infused per minute have been corrected by by subtracting the amount metabolised outside the liver (— indicates that the constant extra hepatic metabolism exceeds the amount infused)*

Cat no	Hepatic plasma flow (ml/min)	Correction factor	Ethanol (mg/l plasma)		Ethanol (mg/min)	
			Uncorr	Corr	Uncorr	Corr
1	107	1 18	12 6	14 9	2 13	1 35
			33 1	39 1	4 33	3 55
2	70	0 77	9 8	7 5	1 20	0 14
			12 6	9 7	1 94	0 88
			20 8	16 0	2.51	1 45
5	74	0 81	2 5	2 0	0 44	—
			5.2	4.2	0 88	—
			12 1	9 8	1 82	0 89
			25 3	20 5	2 79	1 86
6	96	1 05	5 8	6 1	0 86	0 07
			13 1	13 8	1 81	1 02
			17 7	18 6	2.54	1 75
			29 1	30 6	3 48	2 69
			39 4	41 4	4 60	3 81
7	65	0 71	7 9	5 6	1 08	—
			15 8	11 2	2 19	1 03
			31 9	22 6	3 24	2 08
8	48	0 53	7 0	3 7	0 96	—
			14 3	7 6	2 10	0 68
			35 3	18 7	3 10	1 68
10	73	0 80	22 2	17 8	2 86	1 67
			36 8	29 4	3 77	.59
			52 5	42 0	5 03	3 84
11	92	1 01	11 5	11 6	2 01	1.21
			24 2	24 4	2 81	2 01
			34 9	35 2	4 01	3 21
12	113	1 24	23 6	29 3	2 86	2 68
			31 4	38 9	3 77	3.59
			42 7	52 9	5 03	4 85
13	173	1 90	10 6	20 1	2 79	1 80
			16 4	31 [illegible]	3 86	2 87
			22 7	43 1	4 91	3 92

Mean value 91



F g 3 Mg ethanol infused per minut in relat on to th orr sponding a ter al concent ation Th values from the d f ferent exper ments ar plotted in the s me graph after orrect on for d ffer enc in the l er flow and extr hepat c eliminati n The line drawn through th points is the r gression l ne

the 0 point 2) Since a difference in blood flow from one animal to another will give a different slope of the lines the concentrations in each experiment have been multiplied by a factor calculated by dividing the blood flow in the experiment by the mean flow from all the experiments (Table II) After this correction the slope of the different lines should be the same With these two corrections all the points will fall on a straight line passing through the 0 point provided the liver blood flow in the single experiment has been constant The results are illustrated in Fig 3 The line drawn through the points is the regres sion line calculated by the method of the least squares It passes through the 0 point and the complete linear dependence is expres ed by a correlation coefficient of 1 0

The flow values are listed in Table III Flow was calculated using the correction for the amount of ethanol eliminated outside the liver determined both indirectly and directly

A comparison with the flow rates measured on the same cats after a single injection of ethanol (LARSEN 1963 Table I) shows that the flow rate is found somewhat lower when determined by the method of continuous infusion A possible explanation of this fact will be discussed later in this paper

The extrahepatic elimination The values for the amount of ethanol eliminated outside the liver are listed in Table I

In Fig 4 a typical experiment with determination of the elimination of ethanol in the eviscerated cat is illustrated Sixty minutes after the injection the elimination curve is rectilinear and the slight but definite fall indicates

results are listed in Table IV It is evident that the use of ether + nitrous oxide reduces the hepatic plasma flow whereas chloralose has no obvious influence on the flow Apart from cat no 20 the amount of ethanol eliminated outside the liver was found to be reproducible

Discussion

Extraction of ethanol in the liver The results have shown that almost complete extraction of ethanol takes place in the liver in the concentration range used The small amounts of ethanol found in the liver veins may be explained by contamination with blood from the inferior vena cava or afflux of small amounts of blood shunted past the liver cells (blood from capsule and/or connective tissue) A relationship between the amount infused and the concentration in the plasma found in the infusion experiments would certainly have been seen if the extraction was incomplete providing the extraction was constant at different concentrations This is very unlikely and by no means supported by the experimental findings However a constant extraction at different concentrations might be expected in cases where a constant portion of the blood passing through the liver is without contact with functioning liver cells

The extrahepatic elimination It cannot be said to have been definitely proved that the parallel displacement of the curve in the infusion experiments is caused by an extrahepatic elimination of ethanol but the results render it very probable Even a constant incomplete extraction will not influence the calculation of the extrahepatic elimination but will only alter the estimated blood flow (with the experiment in Fig 1 as an example it means that the point of intersection with the abscissa would be the same but the slope of the curve different) The correlation between the amount determined indirectly and directly is not very satisfactory However it can safely be said that the experiments on eviscerated cats have proved an extrahepatic elimination of ethanol of the same order as that found by the infusion method The amount eliminated outside the liver seems to be smaller in the eviscerated cat than determined by the infusion method This is not surprising as the metabolic processes might well be diminished in the eviscerated animal Furthermore it may be that oxidation of ethanol can take place in the organs removed *e g* the intestines Finally a day to day variation in the amount metabolized outside the liver may contribute to the lack of correlation though the few experiments summarized in Table IV are against this

It has not been possible to demonstrate any relation between body weight and the amount of ethanol eliminated outside the liver Neither was any relation found between the weight of the kidneys and the amount metabolized by them

The question as to where the extrahepatic elimination takes place has not been investigated with exception of the amount metabolized in the kidneys

It has been found that on an average 35 per cent of the extrahepatic elimination takes place there (Table I)

The liver is the dominating organ in which the first step of oxidation takes place This has been proved by liver perfusion experiments performed by FIESSINGER BENARD Courtial and DERMIER (1936) COURTIAL (1936 p 34—36) and LUNDSGAARD (1938) Furthermore this was confirmed when the enzyme alcoholdehydrogenase which is responsible for the first step of oxydation was isolated and crystallized from horse liver by BONNICHSEN and WASSEN (1948) The total amount found (1 g/kg liver) is sufficient for oxidizing the amount of ethanol metabolized in vivo (THEORELL and BONNICHSEN 1951)

For many years it has been debated whether the first step of oxidation could take place outside the liver as reviewed by CASIER and DELAUNOIS (1947 p 70—71) JACOBSEN (1952) and ELBEL and SCHLEYER (1956 p 54—56) However experiments in recent years demonstrate clearly that ethanol can be oxidized by organs other than the liver Especially convincing are the experiments where labeled ethanol is used In this way BARLETT and BARNET (1949) found in in vitro experiments that ethanol was oxidized by kidney heart and diaphragm from rats but not by brain tissue MASORO ABRAMOWITCH and BIRCHARD (1953) confirmed these findings and furthermore found a slight oxidation in lung tissue but no oxidation could be demonstrated in striated muscle A definite oxidation was also found in kidney slices by MASORO and ABRAMOWITCH (1954) In 1958 FORSANDER RAIHA and SLOMALAINEN published perfusion experiments using liver and hindlimb preparations from rats and found the hindlimb capable of oxidizing ethanol HEBBELINCK (1959) found an increased excretion of labeled CO_2 in mice during muscular work and accepted this as evidence of oxidation of ethanol by the muscle This conclusion may be incorrect as the result could be explained by an increased oxidation of labeled ethanol metabolites

Using non labeled ethanol SUTHERLAND HINE and BURBRIDGE (1956) BEER and QUASTEL (1958) and WAHLGREN and KULONEN (1960) found an increased uptake of oxygen when ethanol was added to brain tissue (from rats) stimulated by KCl or an electric current In the last two publications where the concentration of ethanol was followed no oxidation of ethanol could be demonstrated Some oxidation however was found by BURBRIDGE SUTHERLAND HINE and SIMON (1959) in non stimulated brain tissue from the rat

These experiments give no information about the total amount of ethanol eliminated outside the liver in vivo but experiments have been performed in eviscerated animals in order to estimate this value MIRSKY and NELSON (1939) found no elimination at all in the eviscerated rabbit whereas LUNDSGAARD (1938) found an elimination in the eviscerated cat which however did not exceed 10 per cent of the elimination in the intact animal CLARCK et al (1940) found an elimination in the eviscerated dog amounting to 19 and 31 per cent of the amount eliminated in control animals

The liver blood flow In the literature it has only been possible to find two reports on the hepatic blood flow in cats In the first of these reports (SCHMID 1908) the blood flow through the portal vein was measured by means of Hurthle's Stromuhr and in five experiments the flow varied between 12 6—19 0 ml/kg/min In the second report (BARCROFT and SHORE 1912) the blood flow was determined directly and ranged between 15—45 ml/min (The body weight was not recorded) Because of the technique used the values are not comparable to the values obtained by the ethanol method and therefore it was chosen to compare the blood flow found in cats with the blood flow in dogs determined by the Fick principle

The liver blood flow in dogs has often been determined since the method of BRADLEY *et al* was introduced in 1945 Like the ethanol method it is based upon the Fick principle using the dye bromsulphalein The extrahepatic loss of bromsulphalein seems to be about 5 per cent of the total amount eliminated (BRADLEY 1960) SELKURT (1953) has compared the bromsulphalein method with the direct determination of the blood flow and found the bromsulphalein value to be 7 3 per cent higher

The liver blood flow has also been determined by the urea output (LIPSCOMB and CRANDALL 1947) and ROSE BENGAL (SAPIRSTEIN and SIMPSON 1955 and COMBES *et al* 1956) COMBES (1960) has made simultaneous determination of the liver blood flow by means of bromsulphalein and ROSE BENGAL and the ratio between the two values was 0 995 Finally Indocyanine has been used for determination of the liver blood flow (KETTERER WIELAND and RAPAPORT 1960) An extensive review of the different methods is given by FISHER *et al* (1956) When the values of the liver blood flow obtained by the methods mentioned above are pooled a mean value of 38 2 ml/kg/min is found (s d 7 6 range 27 6—56 0 ml/kg/min n = 21)

The influence of anesthesia on the liver blood flow The influence of anesthesia on the liver blood flow in dogs has been reported by several authors PRATT HOLMES and SHEID (1952) have determined the blood flow immediately before and during the use of different anesthetics Nembutal and cyclopropane caused either no alteration or a slight increase in blood flow whereas ether decreased the flow (no absolute values were given) GILLMORE (1958) could not demonstrate any significant influence of Nembutal on the blood flow which was 34 5 ml/kg/min before and 30 9 ml/kg/min after four hours of anesthesia This was confirmed by EVRINGHAM BRENNEMAN and HORVATH (1959) In these three papers each dog served as its own control When chloralose was used the blood flow was found to be 47 ml/kg/min (HAMRICH and MYERS 1955) FISHER *et al* (1956) have determined the liver blood flow in three groups of dogs In the first group no anesthesia was given and the blood flow was 46 ml/kg/min In the second group anesthetized with Nembutal the blood flow was 49 ml/kg/min but in the last group anesthetized with ether the blood flow was 70 ml/kg/min

From these results it is seen that Nembutal apparently does not influence the liver blood flow in dogs and the same seems to be the case with chloralose This is in agreement with the results of the experiments on cats reported here The influence of ether on the liver blood flow in cats in the present series is in accordance with the results of PRATT HOLMES and SHEID (1952) but in contrast to what was found by FISHER *et al* (1956)

Conclusion The mean hepatic blood flow in cats anesthetized with Nembutal was found to be 34 3 ml/kg/min when a single injection of ethanol was used for the determination (LARSEN 1963) and 26 8 ml/kg/min when determined by continuous infusion The values are not significantly different but in experiments in humans the results given by the single injection method were also higher (1318 ml/min and 1117 ml/min respectively) (LARSEN 1959 a) This can be explained by the big solvent space for ethanol in combination with the constant extrahepatic elimination which will make the elimination curve after a single injection flatten very slowly The point of deviation from the rectilinear course may then easily be underestimated and the blood flow therefore overestimated This source of error will of course not play any role when continuous infusion of ethanol is used The value given by the infusion method is just below the range of values given by other methods in experiments on dogs but it seems justified to assume that the value found represents the actual liver blood flow in cats

The results have shown that the amount of ethanol eliminated outside the liver cannot be deduced from the body weight Therefore the infusion method must be the method of choice for determination of the liver blood flow by means of ethanol In order to calculate the hepatic blood flow the infusion rate must be changed at least once Sixty minutes are required before complete distribution has occurred and if two samples are taken at each level with fifteen minutes interval the determination of the blood flow can be made within three hours

This method has the obvious advantage that catheterization of the liver can be avoided On the other hand the solvent space for ethanol is greater than that for bromsulphalein indocyanine and Rose Bengal and this makes the method less sensitive for small and quick alterations in flow

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order to forward the processes For each Mol glycerol phosphorylated one Mol DPNH is formed The amount of DPNH was determ ned on a Beckman Spectrophotometer (DU) at 340 nm and from this figure the amount of glycerol was calculated

Reagents Buffer solution 20 8 g hydrazinhydrate 24 per cent
1 5 g glycine
0.2 ml $MgCl_2$ 0 2 M
($MgCl$ $6H_2O$.203)
0 8 ml HCl (conc)
water is added to a final volume of 100 ml
and the pH is adjusted to 9 1
Perchloric acid 30 per cent (w/v)
DPN (reinst)
ATP
GDH (c. 3 000 units/mg protein)
GK (c 85 units/mg protein)
(the enzymes used were produced by Boehringer) ⁄

T chnique 1 5 ml blood was collected in a heparinized tube and centrifuged 0 5 ml plasma was then deproteinized with 0 1 ml perchloric acid 0 1 ml of the supernatant was diluted with 0 2 ml of water and 0 04 ml of the d lution used for determination of concentrations of glycerol between 200—1 000 mg/l At concentrat ons below 200 mg/l plasma 0 1 ml of the undiluted supernatant was used

Immediately before determination the buffer solution and the enzymes were mixed in the following proportions 1 ml buffer/0 33 mg DPN-0 68 mg ATP-0 005 ml GDH and 0 0075 ml GK For each reading 2 ml of the buffer mixture is required The mixture was distributed in 2 ml portions in 6 ml test tubes and the enzymatic processes started by adding the supernatant The test tubes were gently shaken and left undisturbed fo 45 minutes when the amount of DPNH formed was measured

In each determination a water blind and 2 glycerol standards were treated in exactly the same way as the plasma samples and by means of these standards a facto was calculated by wh ch the extinction values were converted to glycerol values

Accuracy and sp fic ty f the method The modif cation used for determ nation of glycerol concentrat ons between 200—1 000 mg/l has a coeffic ent of variat on of 0 94 per cent and the recovery was 98 2 per cent (n = 10) The modif cation used for concentrations below 200 mg/l has a coefficient of variat on of 0 65 per cent and the recovery was 98 5 per cent (n = 10

In order to ensure that equimolar reactions took place the theoretical value of the ext nct on was calculated based upon the molecular ext nction coefficient of DPNH of 6 22 × 10⁶ (HORECKER and KORNBERG 1948) At h gh concent ations 96 6 per cent of the theor t cal alue was found and at small con entrations 96 4 per cent

The method is ery specif c and apart from glycerol only L-a-GP will g ve a reaction. However th compound has never been found in the blood of rats and humans under normal cond tions (WIELAND 1957 Other methods used for determination of glycerol have been tho ughl ev wed by MINER and DALTON (1953 p 167—234)

Comment on the method In cooperat on with Dr Chr C one (researc assistant, Institute of Med cal Phy ology the method publish d by W eland has been lightly modified 1 Wieland suggests that the perchlor ions must be emoved after de proteinization by add ng KOH as this ion will nhibit the enzymatic p ocesses This could not be conf rmed and therefore this step wa omitted 2) The pH of 9 8 of the buffer solut on used by Wieland has in our hands been found to cause a very high extinction of the water blind By changing the pH to 9 1 the extinct on was reduced to a minimum of 0 1 apparently without measurable nfluence on the enzymat c processes

Table I. Values determined from the elimination curve following a single injection of 0.8—0.9 g glycerol/kg body weight. In cat no. 1 the elimination curve was not followed long enough to show the flattening

Cat no.	Weight (kg)	r value	Elimination rate		Hepatic plasma flow (ml/kg/min)	Point of flattening of the elimination curve (mg/l)
			mg/kg/h	mg/min		
1	3.14	0.56	184.8	9.67	—	—
2	2.10	0.64	300.8	10.53	50.1	100
3	2.58	0.67	239.[illegible]	10.29	31.9	125
4	2.14	0.71	305.3	10.89	33.9	150
	Mean value	0.65	257.5	10.35	38.6	125
			Hepatic blood flow		44.4	

The condition of the cats. The experiments were performed on cats in the postabsorptive state anesthetized with Nembutal. The initial dose was given intraperitoneally (30 mg/kg) and anesthesia maintained by sustaining doses given intraarterially so that light, constant, surgical anesthesia was obtained. In order to avoid any loss of glycerol in the urine the ureters on both sides were ligated close to the kidneys in all the experiments. The operation was performed retroperitoneally through an incision in the flanks. The blood pressure was controlled during the entire experiment and the temperature kept constant at 38 degrees (C).

Collection of blood samples. Arterial samples were used taken through a catheter placed in the femoral artery.

Experiment with single injection of glycerol. In four experiments 0.8—0.9 g glycerol/kg body weight was injected and the elimination curve studied by determination of the glycerol content in blood samples taken at intervals of 15—20 minutes.

Experiment with continuous infusion of glycerol. In three experiments a solution of glycerol in isotonic NaCl was infused by means of a motor driven syringe at a rate of 0.187 ml/min. The amount infused per minute was well below the amount eliminated in the single injection experiments to complete saturation of the liver. During each experiment the concentration of glycerol in the solution infused was augmented in order to obtain the different concentration levels of glycerol in the blood. A priming dose was given at the start of the infusion and when the amount infused was changed. This dose was calculated from the weight of the cat and the mean value of r found in the single injection experiments. (The r value represents the part of the body in which glycerol is dissolved to the same extent as in the plasma.)

Experiment with hepatectomy. In five experiments hepatectomy was performed as a two-step procedure as described in detail previously (LARSEN 1963 b). A single injection of 2 g of glycerol was given and the elimination curve studied. The initial concentration of glycerol was about 800—1 000 mg/l plasma. The kidneys were left in situ with the ureters ligated and removed later in the experiment except in one case where they were removed from the start. During the experiment glucose was infused at a rate of 100 mg/kg/hour in order to substitute the normal output of glucose from the liver.

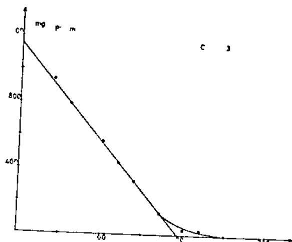


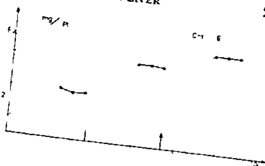
Fig 1 T l m n t n c n f l lo i g a gl j ct on of 1 99 g glycer l

Results

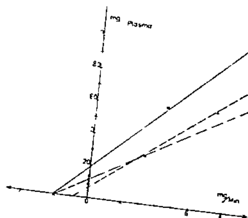
1 *Experiments with single injection of glycerol* The results of these experiments are listed in Table I and a typical elimination curve is illustrated in Fig 1 As the elimination curve for ethanol this curve can be divided into three parts In the first part the fall in concentration is caused by elimination and distribution of glycerol in the solvent space This part ends about 40 min after the injection and the curve becomes rectilinear illustrating that the elimination is constant independent of the concentration At a concentration about 125 mg/l the elimination curve suddenly flattens and gradually approaches the abscissa This flattening may be explained by assuming that the glycerol oxidizing capacity of the liver is no longer saturated and that total extraction takes place in the liver at these small concentrations as has been demonstrated with ethanol

Provided that no glycerol is eliminated outside the liver the hepatic plasma flow may then be calculated by dividing the amount eliminated per minute at complete saturation of the liver by the concentration at which the elimination curve starts to flatten The results are listed in Table I In one case the elimination curve was not followed long enough to demonstrate the flattening of the curve

Only plasma concentrations have been determined and the flow calculated is termed the plasma flow It is not identical with the plasma flow calculated by means of compounds bound to the plasma proteins *e g* bromsulphalein Glycerol is distributed equally in the water phase of plasma and erythrocytes and the plasma flow calculated from the glycerol content in plasma is therefore greater than the actual plasma flow The values have been converted to blood flow by multiplication by the factor 100/100—0 4*H* where *H* is the mean haematocrit (33 4) from ten cats chosen at random



Fg ? Th a t n l co c trat on of gl ce l ft 60 m nut of fu n of gly l At th arro th rr nt fused per m t gment d



Fg 3 Mg gly l infused pe m nut lat o to th o espond ng a t al nt t n f glyc er l Th po ts o th d n e th fast ng al es Th l d wn th gh ach set f po nts is th egress l e (○) = Cat No 5 × = Cat No 6 ● = Cat No 7

B Experiments with continuous infusion of glycerol An experiment is illustrated in Fig 2 Sixty minutes after the start of the infusion and 60 minutes after changing the amount infused the arterial concentration is almost constant indicating that the amount eliminated equals the amount infused As mentioned earlier the amounts infused per minute are well below the amount eliminated at complete saturation of the liver in the injection experiments It has therefore been assumed that complete extraction takes place in the liver and that the arterial concentration represents the arterio hepatic venous difference

Provided that glycerol is metabolized exclusively in the liver and that no glycerol is produced the arterial concentration at equilibrium must be proportional with the amount infused Thus if the results are plotted in a graph with the amount infused per minute as the abscissa and the concentration as the ordinate the points should lie on a straight line passing through the O point

The results of the three experiments are illustrated in Fig 3 The line drawn through the points is the regression line calculated by the method of the least squares The high correlation coefficient (Table II) indicates that the points represent a straight line The lines are all intersecting the ordinate and the

Table II Results from experiments with continuous infusion of glycerol The amount infused per minute was changed twice in each experiment

Cat no	Glycerol infused (mg/min)	Arterial concentration (mg/l)	Point of intersecting of regression line with the ordinate (mg/l)	Fasting value (mg/l)	Constant amount of glycerol metabolized in the liver before infusion (a) (mg/min)	Hepatic plasma flow calculated $\frac{\text{mg/min} + a}{\text{mg/l}}$ (ml/kg/min)	Correlation coefficient
5	3 23 5 55 7 73	31 90 40 96 58 53	11 32	10 34	1 92	64 4 74 0 66 0	0 98
6	3 18 5.28 7.20	29 42 49 87 60 09	6 47	11 41	0 84	49.5 44 4 48 4	0 93
7	4.23 6 04 7 57	60 59 72.30 92 38	18 98	8 23	2 01	32 9 35 5 33 2	0 98
		Mean value	12.25	9 99	1 59	49 8	
					Hepatic blood flow	57 2	

Table III Values determined from the elimination curves following a single injection of 2 g glycerol in eviscerated cats

Cat no	Weight (kg)	k value	Elimination rate	
			mg/kg/h	mg/min
8	4 02	0 58	58 0	3 89
9	3 69	0 55	24 8	1 52
10	3 94	0 66	16 5	1 08
11	4 00	0 [illegible]0	45 5	3 03
	Mean	0 62	36 2	2 38

point of intersection with the abscissa is to the left of the origin This parallel displacement of the line might be explained by a constant oxidation of glycerol in the liver of the fasting cat which equals a constant production of glycerol This amount is represented by the numerical value of the point where the regression line intersects the abscissa Whether this amount is identical with the total amount of glycerol produced in the organism will depend on the existence of any extrahepatic elimination of glycerol This possibility will be discussed later in this article

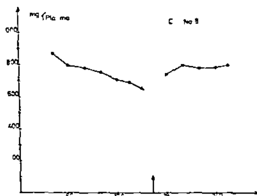


Fig 4 Th l minat n curv foll wing a singl i ject on of 2 13 g glyc rol in an evisc ted cat At the ar w th k dneys are rem ved

However when the amount of glycerol oxidized in the liver in the fasting state is known it is possible to calculate the hepatic plasma flow by means of continuous infusion of glycerol by adding this amount to the amounts infused and dividing the results by the arterial concentrations at equilibrium The plasma flow calculated this way is listed in Table II together with the fasting value and the amount of glycerol oxidized in the liver before the infusion

C Determ nation of the elimination rate in eviscerated cats The results are listed in Table III The r values signify that normal distribution of glycerol takes place in the eviscerated cat With the kidneys in situ a considerable constant elimination of glycerol can be computed from the curves averaging 2 38 mg/min

In three experiments where the kidneys were removed a peculiar rise in plasma concentration of about 100 mg/l was seen After 20—30 min the concentration became almost constant An experiment is illustrated in Fig 4 In one experiment the increa e in concentration was of the same order but took place gradually from the removal of the kidneys and during the rest of the experiment lasting two hours In the experiment where the kidneys were removed from the start the arterial concentration was decreasing very slightly from 60—120 min and thereafter the concentration was completely constant

Discussion

The r value The r value (the part of the body in which glycerol is dissolved to the same extent as in plasma) from the experiments with single injection of glycerol in intact cats and in the eviscerated animals gave a mean value of 0 63 (s d 0 06 n = 8) which is not significantly different from the values for ethanol In five experiments on cats Holst (1944) found a mean value of 0 52 and in experiments on humans the same value was found His r values were based on the glycerol content in whole blood which makes the difference between his values and the values in the present experiments even greater

Table II Results from experiments with continuous infusion of glycerol The amount infused per minute was changed twice in each experiment

Cat no	Glycerol infused (mg/min)	Arterial concentration (mg/l)	Point of intersecting of regression line with the ordinate (mg/l)	Fasting value (mg/l)	Constant amount of glycerol metabolized in the liver before infusion (a) (mg/min)	Hepatic plasma flow calculated as mg/min + a / mg/l (ml/kg/min)	Correlation coefficient
5	3.23	31.96	11.32	10.34	1.9[illegible]	64.4	0.98
	5.5[illegible]	40.96				74.0	
	7.73	58.53				66.0	
6	3.18	29.4[illegible]	6.47	11.41	0.84	49.5	0.99
	5.28	49.87				44.4	
	7.20	60.09				48.4	
7	4.23	60.59	18.93	8.23	[illegible].01	32.9	0.98
	6.04	72.30				35.5	
	7.57	92.38				33.2	
		Mean value	12.25	9.99	1.59	49.8	
					Hepatic blood flow	[illegible]7.2	

Table III Values determined from the elimination curve following a single injection of 2 g glycerol in ... cats

Cat no.	Weight (kg)	"r" value	Elimination rate	
			mg/kg/h	mg/min
8	4.07	0.[illegible]8	[illegible]8.0	3.89
9	3.69	0.55	[illegible]4.8	1.5
10	3.94	0.6[illegible]	16.5	1.0[illegible]
11	4.00	0.70	4[illegible].5	3.03
	Mean	0.6[illegible]	6.2	2.38

point of intersection with the abscissa is to the left of the origin Thus parallel displacement of the line might be explained by a constant oxidation of glycerol in the liver of the fasting cat which equals a constant production of glycerol This amount is represented by the numerical value of the point where the regression line intersects the abscissa Whether this amount is identical with the total amount of glycerol produced in the organism will depend on the existence of any extrahepatic elimination of glycerol This possibility will be discussed later in this article

At equilibrium the amount eliminated is equal to the amount infused plus the amount produced Provided the extraction in the liver is complete in the concentration range used (as indicated by the single injection experiments) the amount metabolized in the liver is proportional to the concentration The rectilinear relationship between the concentration at equilibrium and the amount infused then shows that the resultant of production and extrahepatic elimination is constant and independent of the concentration This was to be expected for the following reasons 1) It is unlikely that the production should be proportional to the concentration 2) It is reasonable to assume that the capacity of the extrahepatic oxidizing system is saturated and the extrahepatic metabolism constant also at small concentrations 3) Thus both the production and the amount oxidized outside the liver may be assumed to be constant

The liver plasma flow (EHPF) can then be calculated from the following equations

$a + p = \text{EHPF} \times c + k$

$a + p = \text{EHPF} \times c + k$

where c and c are the constant arterial concentrations obtained by infusion of a and a mg glycerol per minute p is the constant production and k the constant extrahepatic elimination By subtracting the two equations p and k are eliminated and the liver plasma flow can then easily be calculated In other words the liver plasma flow can be calculated by dividing the difference between the amounts infused by the difference between the constant concentrations obtained It may be noticed that neither p nor k can be calculated by the infusion experiments

The fasting value is only placed on the straight line drawn through the experimental points if the extrahepatic oxidizing system is saturated in the fasting state In Table II the fasting value from the three experiments are listed together with the values found from the point of intersection of the ordinate with the regression line In case 5 the two values are almost identical and in case 6 the fasting value is higher than the calculated value This indicates that the extrahepatic oxidizing system is respectively saturated and unsaturated in the fasting state Finally in case 7 the fasting value is much smaller than the calculated value This is probably due to an experimental error

Apart from case 7 the findings are in accordance with the amount metabolized in the liver in the fasting state When this amount is great the production must be great and the extrahepatic oxidizing system accordingly saturated and vice versa

The liver plasma flow found in the infusion experiments are very high compared to the values found by using ethanol (mean value 29 8 ml/kg/min range 23 7—42 7) and in case 5 and 6 the values are even beyond this range This might be due to the procedure of ligation of the ureters or caused by cessation of urine ecretion

(EHPF) = Est mated H p t c Plasma Flow

This investigat on ha beer p rtly uf ported by g ants from Ko g Chr stian Den T e des Fond Købmand Odense Johann og Hanne W imann fodt Seedo ff leg t and Stat ns alm ndelige V iden kabsfond Thanks are d e to Mrs I AL DAL for valu ble t chnical a stance

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Determination of Acetylcholinesterase Activity in Normal and Denervated Sympathetic Ganglia of the Cat A Biochemical and Histochemical Comparison

By

B HOLMSTEDT G LUNDGREN and F SJOQVIST

Rec ed 4 J ly 1962

Abstract

HOLMSTEDT B G LUNDGREN and F SJOQVIST *Determinati n f ac t l h line t as a ti ty n n mal and d nervat d sympath tic g gl a f th at A b h mi al and h t h m al mpa ison* A ta physiol scand 1963 57 235—247 — AcChE as determined n normal and p egangl onically den rvated sympathet c ganglia with a tit graph c method The upe ior c rvi al st llate 7th lumbar and 1 t sacral gangl a sho ed h ghe AcChE activit es than the ema n ng pa ave tebral gangl a Espec ally lo AChE values c e obta n d in the p ever t b al gangl a In d nervat on expe iments a conside able d crease n AcChE a t ty was noted The ema n ng a ti ty was proportional to th numbe of h stoch mi ally heavily stain d c ll bodies n th g ngl a investigat d Wher as gangl a containing many su h cells r tained 50 of th total AChE acti ity afte d nervat on (L7 and S1 th orrespond ng f gure for gangl a almost lacking them was bout 20 (super r cerv al gangl on) Thus some cells n the mpathet st m how ng ntense histoch mical stain ng possess gn f antly high AcChE activit es than th rema n ng ones as d t m ned b ochem ally Thes cells have been l nked to a periph e l hol n gi fi n t on Small traces of AChE are found in d e g c as w ll as sensory gangl on cell The data p esented f t with tl lass cal onc pt about the sympath et c being ompo ed of predominantly adren rgic cells ith a rgi ones con nt ated n pecial relay stat ons

In sympathetic ganglia acetylcholinesterase (AcChE) is considered to be localized almost exclusively at the presynaptic site in contradistinction to its postsynaptic localization where it is found in the motor end plate. This concept is based predominantly on biochemical determinations of AcChE in the superior cervical ganglion before and after preganglionic denervation. Following this type of operation about 85 % of its total AcChE is lost within the first two weeks (SAWYER and HOLLINSHEAD 1945). These findings were later confirmed histochemically by KOELLE (1951) who demonstrated that most of the AcChE in this particular ganglion was lost after denervation. However persisting activity was found in the neurons, particularly in the cell bodies. Some of these showed intense histochemical staining whereas the majority possessed insignificant activity. The frequency of the markedly AcChE active cell bodies was found later to be highly different in various ganglia (HOLMSTEDT and SJÖQVIST 1959). Further experiments have shown that the heavily stained cells are located almost exclusively in those ganglia which give rise to the postganglionic outflow of cholinergic secretory fibres that innervate the eccrine sweat glands of the cat's paws (viz. stellate and L6-S2; SJÖQVIST 1962 a, b).

The purpose of the present work was to determine whether the histochemical findings could be correlated with corresponding biochemically measurable differences in AcChE activity. As stated earlier (HOLMSTEDT and SJÖQVIST 1961) every histochemical technique ought to be firmly rooted in the use of such biochemical controls. In addition, the combined biochemical and histochemical approach may yield results that are more revealing than any one technique used alone.

By using both normal and preganglionically denervated sympathetic ganglia it is possible to distinguish between pre- and postsynaptically located enzyme (KOELLE 1951; FREDRICSSON and SJÖQVIST 1962). The relative amount of postsynaptic enzyme should be proportional, theoretically, to the frequency of heavily stained ganglion cells, provided that an intense staining really reflects high enzyme activity. It is most feasible to compare the two methods in the lumbosacral ganglia (L5-S2) in which the frequency of heavily stained cell bodies varies between 0 and 13 % of the cell population (SJÖQVIST 1962 a, b).

Material and methods

A. Dissection and operative procedures

The biochemical experiments were performed on sympathetic ganglia of the cat. For purposes of comparison, some nodose ganglia were included. All cats were anesthetized with sodium pentobarbital (Nembutal Abbott 40 mg/kg i. p.).

Acetylcholinesterase activity was determined in the superior cervical, the stellate and the prevertebral ganglia of about 10 cats. The ganglia were dissected free from adjacent pre- and postganglionic fibres. In a few experiments the cardiac zone

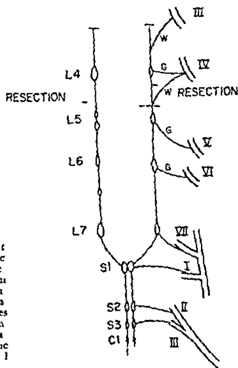


Fig 1 Schematic drawing of the arrangement of the lower lumbar and sacral sympathetic chain and its ganglia. W and G white (preganglionic) and grey (postganglionic) rami communicantes respectively III—VII lumbar spinal nerves I—III sacral spinal nerves The direction of the grey ramus determines the enumeration of the ganglion White rami are generally lacking below the fourth lumbar spinal nerve Consequently no preganglionic fibres enter the chain below the indicated level of resection

of the stellate ganglion *i e* the ventromedial part from which the cardiac nerves arise was dissected free from the remaining non cardiac part The aforementioned ganglia are well defined anatomically and easily distinguished from the fibres contrary to those of the lumbosacral region In the latter case the visible ganglionic swellings of the chain were dissected free from the presumed pre- and postganglionic fibres

The anatomical literature concerning the lumbosacral ganglia of the cat is meager and somewhat unreliable with a few exceptions (LANGLEY 1891 BOTÁR 1932) Dissection in about 40 cats have shown that the sympathetic chain in general contains seven lumbar ganglia three sacral ganglia and a varying number of coccygeal ganglia as described by BOTÁR (Fig 1) When referring to the ganglia LANGLEY's nomenclature has been used Thus the ganglion which sends its grey ramus to the 7th lumbar spinal nerve is called the 7th lumbar sympathetic ganglion etc The ganglia in the lumbosacral chain are not always symmetrically arranged In some cases a contralateral ganglion may be missing in other cases ganglia may be duplicated or fused and lastly the nerve trunk itself may contain ganglion cells especially in the region L6 S? The left and right 1st sacral ganglia are often fused to a single medial ganglion and cannot be denervated by a unilateral sectioning of the chain (Fig 1) The ganglia below S1 are extremely small

Preganglionic denervation of all ganglia below L4 was obtained mostly by resecting a 1 cm segment of the chain between L4 and L5 bilaterally since the white rami generally do not pass out below the fourth lumbar nerve (LANGLEY 1892) However as described by LANGLEY white rami sometimes arise from the fifth lumbar nerve In such cases a resection was made also between L5 and L6

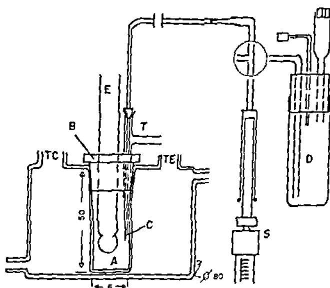


Fig 2 Apparatus fo biochemical determ nat n of ChE A = Glass react on vessel w th th r mostat B = Teflon topper w th electrode T tube and ex t N_2 C = Polyethylene t b g with glass cap llary 0 2 mm. D = Container fo NaOH E = Combination electrode S = Agla m crometer syr nge T = T tube for polyethyle ie tub ng from Agla micrometer syr ng and inlet N TC = Hole for temperat re compensator TE Hole for thermometer

The operative experiments vere made in 11 cats (22 chains) using an abdomina approach and accepted surgical principles The cut ends were retracted from each other and also turned back upon themselves and a bit muscle interposed between the ends At time of dissect on the ganglia were of normal appearance Regeneration was visible in one case and was excluded in the present series The segments L5 S? i e the visible gangl onic swellings together with the chain above and beneath the ganglion were dissected out 1—3 weeks after the operation assayed for AcChE and compared to the corresponding values in normal cats For comparison the superior cervical ganglion was denervated preganglion cally in 4 cats by resecting a 1 cm segment of the cervical sympathetic trunk at the level of the thyroid gland

B Biochem cal techniq e

Equipment

Apparatus

The apparatus employed for the biochemical analyses (Fig 2) were as follow Titrator Type III 1 c and Titrigraph Type SBR 2c/SBU 1 a (Radiom ter Copenhagen) Electrode Radiometer Type GK 2071 c reaction vessel accord ng to Fig 2 equipped with a magnet c stirrer and kept at 25 C by means of a remot ther mostat (Ultrathermostat Type U 3 Lauda/Tauber West Germany) Ham lto syringe 0 5 ml (HAMILTON Co P O Box 307 Wh ttier Cal USA) All glass s ringes were equipped with polyethylene tubing for the transfer of solut ons

Fig 3 Control of the dener v t n exp riments t th cellular level H stochemi cal staining of A ChE n the 1st sacral gangl on. Cryostat ect ns Thiochol ne m th od I ubat on tim 30 min
To the l ft Normal gangl n Heavily ta ned ll bod es rather dense net w k of tained p egangl on c fibres
To th right Tw weeks after preg ngl onic d ner t on. N t la k of act ty n th m j rity f fib es Pers sting AcChE in som cell bod es and a ons Biochemical act ity (2 34 × 10 μmoles ac d mg min) consequently eflects post synapt c AcChE act ity



The homog n zer used was a sl ght mod f cation of the one descr bed by ALDRIDGE E ERY and STREET (1960) Its inner diameter was 13 mm Th e bottom was made of stainless steel The distance between the pestle and glass tubing was 0 08 mm and the speed of rotation was 900 cycles/ minute

Reagents

The reagents used for A ChE assay were the follo ing aqua dest (deionized and CO_2 free) NaOH 0 00?5 M the NaOH was made up in CO free aqua dest and tested for each set of experiments (ee belo) KCl 0 1 M adjusted to pH 8 with NaOH and kept CO_2 free substrat ac tyl β methylchol ne od de 6 0 × 10^{-2} M (Beta cholyl, Vitrum Stockholm) kept n 0 1 M CO_2-free KCl pH about 6

All olutions used e e bubbled through with washed N before the determination and protected f om a r with soda l me traps N trogen also was blo n slowly over th fluid n the rea t on essel both during the temperature equilibration and the t trat on n order to exclud any nterfer nce by atmospher c carbon dioxide

P oc d

Standardi t on of NaOH solt t on

A volum of 4 5 ml CO f ee aqua dest and 0 ? ml 0 001 M HCl was added to th r action essel By means of th automatic titrato th pH was adjusted to 7 0 with the N OH solut on After equilibrium 0 50 ml of 0 001 M HCl was added by means of th Ham lton syringe Th olume of NaOH equ red for readjustment to pH 7 0 was ecorded t trigraph call on the bscissa where 1 mm corresponds to 0 00? ml of the titrant

Homog n at on

The we ghed ga gl on as homogeni ed in 0 1 M CO_2-free KCl at pH 8 (1—2 mg w t w ght per ml) The homogen t was transferred to a glass-stoppered test tube and bubbled through with washed N for 5 m n

Table I AcChE activity in different ganglia of the cat[1] e pressed in µmoles acid × 10[3] mg[−1] m n[−1]

Ganglion or segment	Number of cat	AcChE in g nglia	Nun ber of cats	AcChE in segment
Nodose	3	3 33 ± 0 69	—	—
Super or cerv cal	—	9 13 ± 1 69	—	—
Left	11	9 09 ± 1 40	—	—
R ght	4	9 ?1 ± 1 06	—	—
Stell te	—	9 30 ± 1 98	—	—
Left	12	9 43 ± 2 00	—	—
Right	4	8 91 ± 1 84	—	—
Card c part	5	6 91 ± 0 89	—	—
Non card ac part	5	9 11 ± 1 19	—	—
Coeliac	8	4 46 ± 1 14	—	—
Superior mesenter c	8	4 06 ± 1 39	—	—
Infer or mesenter c	4	3 08 ± 0 19	—	—
Thorac c[4]	2 (12)	5 50 ± 0 69	1 (10)	3 12 ± 0 65
L1	5 (8)	5 52 ± 0 44	4 (7)	4 89 ± 0 91
L2	5 (8)	5 78 ± 1 04	4 (6)	5 95 ± 1 11
L3	5 (10)	7 20 ± 0 47	6 (11)	6 99 ± 1 37
L4	5 (10)	7 06 ± 0 86	6 (11)	6 54 ± 1 04
L5	5 (10)	7 53 ± 0 94	6 (12)	6 31 ± 1 71
L6	5 (10)	5 33 ± 0 69	6 (12)	5 5? ± 1 67
L7	5 (10)	8 70 ± 2 40	6 (12)	7 66 ± 1 65
S1	5 (7)	8 42 ± 1 42	6 (8)	7 80 ± 2 66
S2	2	4 74	4	5 51 ± 0 76
S3	2	3 60	4	5 88 ± 1 17

The valu s represent means ± S D per cat (S D = $\sqrt{\frac{\Sigma(x-\bar{x})}{n}}$ when n > 9 otherwise the den minator n 1)

The number of gangl a when not id nt cal w th the number of cats is given w th n pa an thes s

For definit on s p ge 4

Th value represents the mean of d fferent thor c g ngl a

Determination of AcChE activity

The enzyme activity was determined in a final volume of 5 ml The homogenate conta ning the known amount of tissue was made up to a volume of 4 5 ml in 0 1 M KCl (pH 8 0) in the react on vessel The magnet c stirrer was started and the Agla m crometer syr nge filled with 0 0025 M NaOH from the storage vessel The titrigraph was then started and the pH adjusted to 8 0 After equilibration 0 5 ml of 6 0 × 10[−] M acetyl β methylcholine was added The paper speed in most experiments was 5 mm/min and 10 min required to obta n a straight line recording

Curves for spontaneous hydrolysis were run in exactly the same solut ons the ganglion tissue excluded The spontaneous hydrol s s if appreciable was subtracted from the enzymic one

Table II AcChE acti ty in d ff rent denervated gangl a of the cat[1] exp essed in µmoles acid × 10[3] mg⁻ min⁻¹

Gangl n o segment	Number of cats (ganglia)	AcChE in g nglia	AcChE in segments	Per ent f ont ol
S perio erv cal	4 (4)	1 94 ± 0 45	—	21.2
L5	10 (20)	—	1 96 ± 0 31	31 1
L6	10 (20)	—	1 99 ± 0 67	36 1
L7	10 (19)	—	3 96 ± 1 09	51 7
S1	10 (12)	—	3 63 ± 0 74	46.5
S	8 (8)	—	1 99 ± 0 61	36 1

The alues ep esent means ± S D per cat
The number f ganglia when not ident c l w th th numbe of cats s gi en w thin p ran thesis.
Cont ol al es f nd in T ble I ght col mn.
The differ n es between L7 and S1 nd the other g nglia a e h ghly s gnificant (t test $P < 0.001$)

The l nearity of the method for the amounts of enzyme used was determ ned in a pecial set of experiments In control tests th difference between dupl cate experiments of the same homogenates amounted to about 1 5

Calculation

The abscissa of the curve as obta ned from the titrigraph nd cates the amount of NaOH in µmole used after nit al cal bration The ordinate indicates time in minutes

Simple read ng and conversion gives the acetylchol nesterase act v ty thus being xpressed as µmole of liberated acid per mg tissue and minute at 25 0 pH 8 0 *t*
AcChE a t vity = µmole acid × mg⁻ min

C H tochem l nt l

H tochemical demonstrat on of AcChE was performed with the th ocholine method as mod fied by Holmstedt (1957) The normal ganglia have be n stud ed ex t nsi ely a l e (Sjoqvist 1962 a b Fredricsson and Sjoqvist 1962) In the p esent tud the effect of preganglionic denervat on was controlled at the cellular level in ertain ganglia of som experimental animals (F g 3) In such cases a small pa t of the gangl on was ex ised fo h stochem cal in est gat on and the rest of it l ft to b o chem cal determ nat on of AcChE

Results

Normal ganglia

Data in Table I demonstrate that the ganglia from some regions of the sympathetic chain differ from the rest in having a higher AcChE activity This pertains especially to the superior cervical the stellate the 7th lumbar

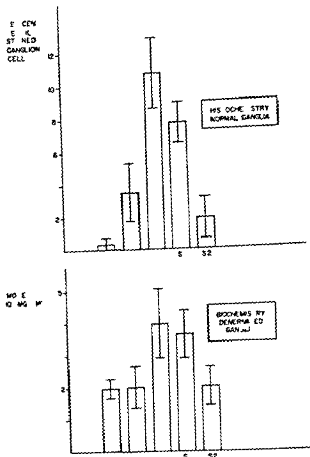


Fig 4 Comp ison between the histochem cal and b o-chem cal methods for d term t on of AcCl E

Abo P cent hea h sta d gangl n cells in d ffe ent normal gangl a (quot d f om Sjoqu t 1962 b)

B low AcChE n μmoles ac d × 10 mg⁻ m n in rrespond ng ganglia afte p egangl on c denervat n

All values g n as me ± S D pe cat

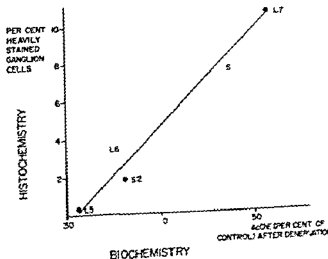


Fig 5 Number of l t n d AcChE h g gl n c ll s d term ed h toch m ally n d ff t gangl a pl tt d g t th AcChE act ty of den ted g gl a p essed p nt of t tal a t

and 1st sacral ganglia The lumbar ganglia L3 L5 have a slightly higher activity than the remaining paravertebral ones In the stellate ganglion the ventromedial portion i e cardiac part (SJOQVIST 1962 a) has a lower activity than the rest The prevertebral ganglia show considerably lower activity than any of the chain ganglia It is also obvious from Table I that the lumbosacral segments analyzed have AcChE activities of the same order of magnitude as the corresponding ganglia dissected free

Denervated gan lia

Preganglionic denervation results in a loss of AcChE from the dense network of preganglionic fibres whereas the activity of the cell bodies is left intact (Fig 3) Some cell bodies are outstanding by showing heavy staining intensity They are especially frequent in the 7th lumbar and 1st sacral ganglia (Fig 4) Statistically these particular ganglia also retain significantly higher AcChE activities after denervation than the other lumbosacral ganglia (see also Table II) Although the latter ones (L5 L6 S2) have varying number of heavily stained cell bodies this difference is not reflected biochemically in absolute values per mg (Fig 4) The remaining AcChE activity in per cent of control is about 50 % in L7 and S1 but only 20 in the superior cervical ganglion and just above that value in other lumbar ganglia (Fig 5 Table II) No appreciable difference in AcChE activity was found between the survival times of 1 2 and 3 weeks following denervation which is in accordance with results obtained by Couteaux and Nachmansohn (1940)

Discussion

When studying the distribution of enzymes by biochemical analysis of nervous tissue homogenates it is almost impossible to predict anything about their cellular localization Thus the determination of cholinacetylase which generally is better correlated to cholinergic transmission than AcChE (HEBB 1961) is hampered by the lack of a histochemical control method On the other hand the histochemical demonstration of AcChE as is apparent some times in recent literature has developed into a purely descriptive histological method without any biochemical controls The evaluation of the results obtained with the thiocholine method is based on the estimation of the relative staining intensity of different tissues

The purpose of the present investigation has been to determine whether the proportion of heavily stained ganglion cells in certain *sympathetic ganglia* really possesses a significantly higher AcChE activity than the rest of the cell population This seemed reasonable since the histochemically outstanding cells have been demonstrated almost exclusively in the sweat secretory ganglia (SJÖQVIST 1962 a b) where cholinergic (DALE and FELDBERG 1934) post ganglionic fibres arise to innervate the eccrine sweat glands of the cat s paws (LANGLEY 1891)

The biochemical control of the histochemical results is performed preferably on preganglionically denervated ganglia since the AcChE activity of the heavily stained cell bodies is located postsynaptically When comparing the absolute AcChE activities of the denervated ganglia L5 S2 differences in the density of ganglion cells may invalidate the comparison (Fig 4) This is however not the case when the AcChE activities are expressed in per cent of total activity (Fig 5) The small differences in AcChE activity obtained between each lumbosacral ganglion and its corresponding segments are due presumably to the occurrence of ganglion cells along the entire length of the trunk in this region

The titrigraphic determination of ChE (LARSSON and HANSEN 1956 JENSEN HOLM *et al* 1959) as outlined by the authors under Material and Methods proved to be a sensitive and accurate means of determining ChE in isolated ganglia weighing even less than 0 5 mg The sensitivity also allowed determinations of ChE in small interganglionic segments and denervated ganglia Earlier biochemical determinations of ChE in sympathetic ganglia have dealt with few exceptions (HOLMSTEDT and SJÖQVIST 1959 McISAAC and KOELLE 1959) almost exclusively with the superior cervical ganglion (COUTEAUX and NACHMANSOHN 1940 SAWYER and HOLLINSHEAD 1945 KOENIG and KOELLE 1961)

This study of ganglionic AcChE is the first one to include all sympathetic ganglia The total enzyme activity pre and postsynaptic was found to be highest in the superior cervical the non cardiac part of the stellate the 7th lumbar and 1st sacral ganglia Only the last three ones contain heavily stained ganglion cells in appreciable numbers demonstrating that no evident correlation exists between the total AcChE activity of a ganglion and its content of heavily stained neurons On the other hand the prevertebral ganglia lacking such neurons showed low activity comparable to that of the nodose ganglion considered to be predominantly sensory (HILLARP 1960)

After preganglionic denervation AcChE disappears from the preganglionic terminals as shown both histochemically and biochemically (COUTEAUX and NACHMANSOHN 1940 SAWYER and HOLLINSHEAD 1945 KOELLE 1951 KOELLE and KOELLE 1959 FREDRICSSON and SJÖQVIST 1962) The remaining activity is confined to AcChE in cell bodies and postganglionic fibres (see Fig 3) Fig 4 and 5 show that the AcChE activity of the denervated lumbosacral ganglia is approximately proportional to their number of heavily stained ganglion cells Whereas L7 and S1 have about 50 % of the total AcChE located presynaptically the corresponding figure for the superior cervical ganglion is 80 % (Table II) which is in agreement with the 85 % figure found by SAWYER and HOLLINSHEAD (1945) Thus the ganglion used "par préférence for biochemical and pharmacological experiments is rather unique by having a very high proportion of its total AcChE located at the presynaptic site This may be explained by its very dense network of preganglionic ter

minations (FREDRICSSON and SJOQVIST 1962) The concept of where no preganglionic fibres no AcChE (SAWYER and HOLLINSHEAD 1945) is no longer tenable

It is apparent that sympathetic ganglia may be more or less cholinergic postsynaptically in the sense that they contain different numbers of heavily stained cells The cells in question must be particularly AcChE-rich since the ganglia containing them in large numbers (L7 and S1) retain significantly higher activities after denervation However ganglia having fewer but varying numbers of such cells (L5 L6 S2) all have the same activity as determined biochemically This may reflect the superiority of the histochemical technique The background activity obtained biochemically may be due partly to the occurrence of intermediately stained ganglion cells which have been found in all paravertebral ganglia (FREDRICSSON and SJÖQVIST 1962) but never constitute more than a few per cent of the total cell population (SJOQVIST 1962 b) They occur with about the same frequency in all chain ganglia which may explain why the background activity is of the same order of magnitude in all sympathetic ganglia investigated after denervation except L7 and S1 (Table II) A certain amount of the background activity is due possibly to traces of AcChE which have been demonstrated histochemically in the majority of adrenergic cells as well as in sensory neurons (FREDRICSSON and SJOQVIST 1962) Finally some activity may be due to persisting red [illegible] in the tissue This raises a point worthy of comment other authors ha[illegible] demonstrated traces of AcChE in most nervous tissue independent of its fu[illegible] tional classification i e cholinergic adrenergic sensory etc (KOELLE [illegible] NACHMANSOHN 1959) As discussed by FELDBERG (1957) there are ma[illegible] sibilities to explain the AcChE activity of non cholinergic nervous t[illegible] present authors however do not view such small activities as evid[illegible] a general cholinergic process at sympathetic postganglionic nerve [illegible] has been proposed recently by BURN and RAND (1960)

As to the function of cytoplasmic cholinesterase many theori[illegible] discussed before (KOELLE 1962) Independently of the questi[illegible] cellular enzyme is transported to the nerve-endings or not (KOE[illegible] 1961) it has been demonstrated that AcChE activities of th[illegible] of the neuron (i e the cell body the axon and the termina[illegible] organ) coincide in a striking way (KOELLE 1955 GIACOBIN[illegible] 1958 GIACOBINI 1959 FREDRICSSON and SJOQVIST 1962) [illegible] that the enzyme of the cell body reflects the peripher[illegible] of the neuron

In summary the results obtained histochemically c[illegible] well with the quantitative biochemical values of AcCh[illegible] Some cells in the sympathetic system are detected ea[illegible] chemical staining These cells have been linked t[illegible] function i e sweat secretion (SJÖQVIST 1962 b) [illegible]

significantly higher AcChE activities than the rest of the cells as determined biochemically The histochemical method therefore may be helpful in identifying cholinergic ganglion cells as defined by DALE (1933) in the sympathetic system of the cat The authors do not draw any conclusions as to the function of the small traces of AcChE found in the majority of adrenergic as well as sensory ganglion cells

The results of the biochemical and histochemical experiments are totally compatible with the classical concept about the sympathetic system *viz* that it is composed of predominantly adrenergic cells with a minority of cholinergic ones exhibiting outstandingly high AcChE activity

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Reflex Responses and Recurrent Discharges Evoked by Stimulation of Laryngeal Nerves

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Abstract

MÅRTENSSON A *Reflex responses and recurrent discharges evoked by stimulation of laryngeal nerves* Acta physiol scand 1963 57 248—269 Reflex responses in intrinsic laryngeal muscles to stimulation of the internal laryngeal nerve were studied with a view to elucidating the mechanism of the protective reflex closure of the glottis Ipsilateral stimulation evoked reflex discharges of multisynaptic character in all four vocal cord adductors studied but never in the abductor in which only inhibition of pre existent activity was observed Contralateral stimulation evoked similar reciprocal effects but the discharges in the adductors were smaller and of longer latencies and did not appear in all muscles latent contralateral reflex connections could however be demonstrated in all muscles The reciprocal innervation pattern and the difference in transmitter potentiality of different reflex connections are discussed 2/ Proprioceptive mechanisms in the laryngeal musculature were investigated Stimulation of individual muscle nerves evoked in nerve and muscle in addition to the direct response a secondary response amounting to a few per cent of the primary response By performing an analysis in analogy with previous work on the spinal cord it was concluded that the secondary response represented a recurrent discharge from the motoneurons whereas no response exhibiting the properties of a monosynaptic reflex was observed Attempts to record afferent impulses from muscle spindles gave negative results The findings are discussed in relation to earlier results from morphological and physiological studies of proprioceptive systems in the larynx

According to NEGUS (1949) the primary function of the larynx from a phylogenic point of view is to act as a sphincter system protecting the lower airways from an intrusion of foreign bodies In higher species where the sound production is the most conspicuous larynx function a protective mechanism still exists in the form of a reflex closure of the glottis elicited by mechanical stimulation of the laryngeal mucosa Studies of this protective reflex have so far chiefly been limited to observations of the vocal cord movements elicitable by adequate or artificial stimulation (KATZENSTEIN 1908 MUNDNICH 1956) Only few attempts have been made to examine how the individual laryngeal muscles are engaged in the glottic closure reflex and to find out what types of reflex mechanisms are involved (YAMASHITA and URABE 1959 1960 cf also DOTY and BOSMA 1956)

The fact that the intrinsic laryngeal muscles are capable of the precise co-ordination necessary for the production of sound has drawn a great deal of attention to the problem whether a proprioceptive feed back exists in this system The controversial views put forward in this question have mainly been founded on histological studies concerning the presence of sensory endings in the muscles While several authors have asserted that no muscle spindles occur in the laryngeal muscles of man and of different animal species (SHERRINGTON 1897 CILIMBARIS 1910 MUNDNICH 1956) such endings have actually been described in later years as existing in varying numbers (*e g* GOERTTLER 1950 PAULSEN 1958 BOWDEN *et al* 1960 LUCAS KEENE 1961)

So far only few physiological investigations have been made to try to find out whether there is a proprioceptive system in the laryngeal muscles (cf BIANCONI and MOLINARI 1960 1961) Attempts to record afferent impulses from separate muscle nerves seem to have been made only on the cricothyroid and the results obtained have been negative both in the rat (ANDREW 1956) and the dog (PAULSEN 1958) In the rat ANDREW (1954) has however recorded afferent impulses from receptors in the thyroepiglottic joint whose discharge frequency varied with movements of the joint and this receptor system was assumed to be engaged in the reflex control of muscles acting on the joint in question

Only one neurophysiological work seems to have been published with the primary aim to demonstrate proprioceptive reflexes in the larynx *viz* by ESSLEN and SCHLOSSHAUER (1960) In connection with total extirpation of the larynx in human cancer cases these authors have excited the intact laryngeal nerves and recorded direct and centrally relayed responses from the different muscles the latter responses were interpreted as being monosynaptic reflexes No animal experiments seem to have been made to establish whether proprioceptive reflexes can be elicited in the intrinsic laryngeal muscles

In this paper the first section will give an account of investigations designed to study the mechanism of the glottic closure reflex in the dog evoked by electrical stimulation of the internal laryngeal nerve it will be shown that the

glottic closure is produced by activation of the vocal cord adductors and inhibition of the abductor via polysynaptic reflex arcs The second part of this work is concerned with the question whether there is a proprioceptive system in the intrinsic laryngeal muscles as will be shown responses of central origin were obtained on stimulation of the separate muscle branches A closer analysis revealed however that these responses do not show the characteristics of monosynaptic reflexes but should be interpreted as recurrent discharges from the motoneurons Nor could any evidence for a proprioceptive system in the intrinsic laryngeal muscles be obtained from attempts to record afferent impulses from the single muscle nerves

Methods

Some 50 dogs weighing 5—8 kg were used in these experiments Anesthesia was induced by nembutal 40 mg per kg bodyweight given intravenously or intraperitoneally and maintained by repeated i v injections of nembutal or thiogenal

The experiments were generally performed only on the left side of the larynx which was exposed as follows A ventral midline incision between the sternal and hyoid bones was extended from its upper end to the left jaw angle and the cranial part of the ipsilateral sternohyoid muscle was excised A tracheal cannula was inserted and the subsequent operative procedure was partly performed with the aid of a low power binocular microscope The sternothyroid muscle was detached from its attachment to the thyroid cartilage and the peripheral part of th n rve to the cricothyroid muscle was dissected f ee down to its po nt of entry into the muscle The internal branch of the superior laryngeal nerve viz the internal laryngeal nerve was dissected free in its extralaryngeal portion The thyrohyoid muscle and the pharyngeal constrictors were cut at their attachments to the laryngeal cartilages and the major part of the thyroid lamina was retracted so as to expose the ntralaryngeal portion of the internal laryngeal nerve After subjecting the cricothyroid to an experimental analysis the muscle was divided and its cranial pa t retracted together with the rest of the thyro d lamina close to the midline thus exposing the other intrinsic laryngeal muscles with their different nerve branches from the lateral side These branches — all deriving from the laryngeal branch of the recurrent nerve — were f nally dissected free as was also the anastomosing branch connecting the nternal and the recurrent laryngeal nerve

In exploratory experiments the different muscles v ere f rst identified and their innervation studied by separate stimulation of the various nerve branches and recordi s from the muscles The following muscles were invest gated viz the cricothyroid the thyroarytenoid the interarytenoid and the lateral and posterior cricoaryteno d The thyroarytenoid as well as the interarytenoid muscles in man are generally described as being composed of two parts and this may of course also apply to the dog however in this investigation no attempt has been made to study them separately

In one series of experiments the stimulating electrodes were placed on the central end of the cut ipsi or contralateral internal laryngeal nerves In some preparations record ngs of the reflex effects v ere made from muscle nerves but in most of the experiments the reflex responses were recorded from the muscles This may of course imply the risk of picking up acti ity from nearby muscles particularly from muscles yielding h gh amplitude reflex responses but satisfactory select vity could be ensured by appropriate denervation of the muscles As a rule th muscles were studied in the following order the cricothyroid the thyroaryteno d the lat ral cricoarytenoid the interarytenoid and finally the posterior cricoarytenoid muscle M ventricularis was not denervated it is

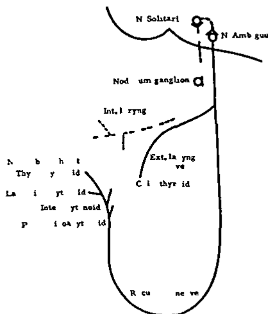


Fig 1 Schematic diagram of peripheral pathways in laryngeal reflexes

like the interarytenoid innervated by a nerve branch running below the posterior cricoarytenoid and a selective denervation of the muscle was considered to be technically complicated and to involve risks of circulatory disturbances In all muscles except the interarytenoid the electrodes were inserted from the lateral side To the interarytenoid access was gained by dissecting free the larynx from the pharynx and from the hyoid bone and then lifting up the organ at the cranial end

In a second series of experiments primary and secondary responses to the individual laryngeal muscles were elicited by stimulation of the corresponding muscle nerve branches The interarytenoid was not included in these experiments since it was considered difficult to activate this muscle selectively in view of its innervation (cf above) As a rule recordings were made from the muscles and only in a few experiments from muscle nerves In connection with these experiments attempts were also made to record afferent impulses from the thyroarytenoid and cricothyroid muscles in response to muscle stretch For this purpose the peripheral end of the cut muscle nerve was split up into filaments which were placed on the recording electrodes

Square wave stimuli of 0 1 msec duration and of varying frequency were used for the stimulation of different nerve branches The reflex activity in the muscles was recorded by means of two steel needles insulated except for the tip Bipolar silver wire electrodes were used for recording from muscle nerves The recording electrodes were connected to a Tektronix 5 [illegible] 0 oscilloscope and a loudspeaker via a cathode follower and an amplifier (Grass P 6)

Results

1 Reflexes elicited by stimulation of the internal laryngeal nerve

The experiments to be described in this section were performed on some twenty five dogs and comprised studies of reflex responses obtained from



Fig 2 Ipsilateral reflex responses to single shock stimulation of internal laryngeal nerve recorded in *A* the nerve to the thyroarytenoid muscle *B* the thyroarytenoid muscle *C* the cricothyroid muscle Time bar 10 msec.

various laryngeal muscles after ipsi or contralateral stimulation of the central end of the cut internal laryngeal nerve (see Fig 1)

Anatomy It has long been known that the internal laryngeal nerve contains afferent fibers from the laryngeal mucosa and it has also been shown by experiments on dogs that this nerve does not supply the laryngeal muscles (LEMERE 1932 VOGEL 1952 confirmed in this investigation) In the rat ANDREW (1954) has demonstrated the presence of afferent fibers from the thyroepiglottic joint in one of the internal laryngeal nerve branches and it seems most likely that this finding should apply also to the dog These fibers are however few in number and most of the fibers in the internal laryngeal nerve extend from the laryngeal mucosa

On touch stimulation of the laryngeal mucosa the glottis is reflexly closed and the same effect is obtained by electrical stimulation of the internal laryngeal nerve (cf MUNDNICH 1956) The muscles engaged in changing the glottic aperture are the four vocal cord adductors the thyroarytenoid the lateral cricoarytenoid the interarytenoid and the cricothyroid and the vocal cord abductor the posterior cricoarytenoid

All the intrinsic laryngeal muscles except the cricothyroid are innervated by the recurrent nerve and the peripheral conduction distance is thus comparatively long measurements on dogs used in the present experiments gave values of 35—45 cm for the left recurrent nerve As regards the innervation of the cricothyroid in the dog contradictory views have been put forward According to VOGEL (1952) this muscle is supplied by the external laryngeal nerve whereas LEMERE (1932) asserted that motor fibers may also reach the muscle via another vagus branch The peripheral conduction distance is about the same in both cases and considerably shorter than for the other muscles or about 8—10 cm

Ipsilateral reflex responses Stimulation was as a rule applied to the central end of the internal laryngeal nerve sectioned at the level of its entry into the larynx

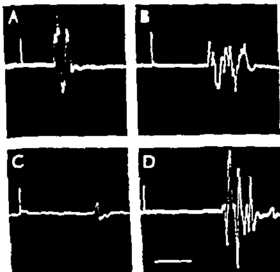


Fig 3 Ipsilateral reflex responses in various intrinsic laryngeal muscles showing typical differences in amplitude and latency *A* cricothyroid, 10 msec latency *B* thyroarytenoid, 16 msec latency *C* interarytenoid 21 msec latency *D* same as *C* during post tetanic facilitation, 2? msec latency (see text) Gain of amplification in *C* 2.5 times that in *A* *B* and *D* Time bar 10 msec.

in some experiments stimulation was also applied to the intralaryngeal nerve branches including the ramus communicans Similar reflex responses were obtained in all cases

A typical ipsilateral reflex response recorded from the nerve to the thyroarytenoid muscle is illustrated in Fig 2 *A* showing an asynchronous discharge of about 7 msec duration of a type similar to a polysynaptic reflex response at the spinal level The latency is 13 msec which represents an average value The central reflex time can only be roughly calculated since the conduction time for the afferent and efferent limbs of the reflex arc can only be approximately assessed in this type of experiments From previous recordings of the afferent volley in the fasciculus solitarius (cf DOTY and BOSMA 1956) the conduction time in the afferent limb of the reflex arc may be estimated at 1—1 5 msec and in special experiments in connection with this investigation the average conduction time in the efferent nerve has been assessed at about 6—7 msec this would give a total central reflex time of at least 4—5 msec which supports the concept of a multisynaptic transmission No definite conclusions can however be drawn about the actual synaptic transmission time until recordings have been made from the central nuclei

Since the absolute latency is thus of less interest in the present investigation and only the relative values have to be considered the reflex effects can be recorded from the muscle which is more convenient since many of the nerve branches are short and difficult to dissect free As appears from Fig 2 *B* the response recorded by needle electrodes in the thyroarytenoid muscle is of the same repetitive type as that obtained in the nerve In the responses recorded from the nerve there were usually spontaneous variations in amplitude and in

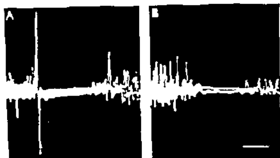


Fig 4 Tonic discharges in posterior cricoarytenoid (upper beam) and cricothyroid (lower beam) varying cyclically with respiration Beginning of expiratory phase at base line deviation in upper tracing Time bar 400 msec

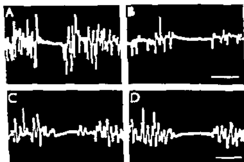
the muscle response this is reflected by variations in its configuration The somewhat longer latency of the muscle response is typical owing to variations in the neuromuscular transmission and in the conduction along the muscle fibers there are greater fluctuations in latency as well as in the duration of the responses

In all the intrinsic laryngeal muscles with adductor function reflex responses of fundamentally the same type as those obtained from the thyroarytenoid could be recorded Fig 2 C shows a response recorded in the cricothyroid The latency is strikingly shorter than in *B* due to the difference in conduction time in the efferent nerves (cf Fig 1) In many experiments reflex responses were less readily obtained from this muscle than from the thyroarytenoid and this was the case also when recording from the other adductors the lateral cricoarytenoid and the interarytenoid muscles In the two latter the reflex responses generally had a shorter duration and lower amplitude than those obtained from the thyroarytenoid Fig 3 illustrates the difference in amplitude between typical reflex responses recorded in the cricothyroid (*A*) the thyroarytenoid (*B*) and the interarytenoid muscles (*C*) The smaller response in *C* is of a significantly longer latency than that in *B* although the peripheral conduction time may be assumed to be about the same for these two muscles which are both innervated by the recurrent laryngeal nerve (cf Fig 1) This also applies to the low amplitude response in the lateral cricoarytenoid The longer latency observed for these two muscles does however not seem to represent any definitely longer central reflex time since the latency may be shortened under certain conditions of facilitation induced *e g* by simultaneous ipsi and contralateral stimulation

As appears from the lower tracing in Fig 4 tonic discharges varying with respiration may be present in the cricothyroid If a reflex is elicited while such activity is going on the reflex response is followed by a silent period in the background discharge (Fig 5 *A*) As a rule this period lasted about 30 msec but in some instances it had a duration of 80 msec In cases of low central excitability or reduced stimulus strength a similar cessation of the tonic ac



Fg 5 4 silent period f llowing e-flex discharge in cr othyro d mu l ev k d by t mulation of psilateral larynge l nerv *B* same phenom n n w thout distinct p c ding reflex disch g Three superimposed sweep in ch reco d Stimulus freq ency 10/ Stimulus art f ts downward in thes eco d Tim bar 10 msec



Fg 6 Inhib t n f pr -exi tent tonic ac tivity in post cricoaryten d muscl ev ked by timulati n of psil ter l internal l ryng al nerv N t d ff nce n d ra ti n of inhib tory ff ct when licited by timul of equal trength during *A* intens and *B* less intens b ckground ti ty and when l ted by *C* weak, and *D* strong timul during ba kground tivity f equal inten ty N t absence of flex discharg Tim bar in *A* and *B* 40 ms in *C* and *D* 20 mse

tivity in the cricothyroid may occur even when no preceding reflex discharges have been discernible in the pre-existent activity (Fig 5 *B*)

In the other adductors such as the thyroarytenoid and the lateral cricoaryte noid muscles no spontaneous tonic activity is present during normal respiration but a background discharge can be set up by repetitive stimulation of the internal laryngeal nerve Also in these muscles the tonic activity thus evoked may be inh ibited after each reflex discharge

On stimulation of the internal laryngeal nerve no reflex discharges similar to those recorded in the adductors were elicited in the vocal cord abductor the posterior cricoarytenoid However in this muscle a spontaneous activity may also be present which — like that in the cricothyroid — increases cyclically with inspiration (Fig 4 upper tracing) and this activity is inhibited by afferent stimulation The inhibition which may last up to 90 msec, may vary in duration not only with the stimulus strength but also with the level of the spontaneous activity In Fig 6 are shown such reflex inhibitions evoked in records *A* and *B* at constant stimulus strength and different amplitudes of the background discharges and in records *C* and *D* at varying stimulus strengths and a constant tonic activity

In the literature on reflex activity it has often been asserted that the natural asynchronous inflow into the central nervous system should be more truly reproduced by repetitive electrical stimulation than by single stimuli As

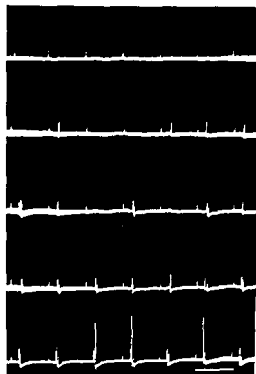
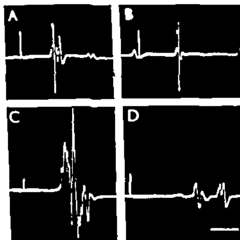


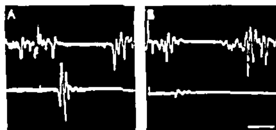
Fig 7 Facilitation of ipsilateral reflex response in the interarytenoid muscle by frequent stimulation (at 10/sec) Four upper tracings continuous recording from onset of stimulation (note small artefacts) a period of 3 sec cut out before lowest record See text Time bar 100 msec

already described, single stimuli gave rise only to feeble reflex responses (Fig 3 *C*) or in some instances to none at all in the lateral cricoarytenoid and the interarytenoid muscles In order to test whether repetitive stimulation would favor the reflex responses in these muscles 5 experiments were performed in which stimuli of a frequency of 10 imp/sec were applied to the internal laryngeal nerve A typical experiment of this kind is illustrated in Fig 7 showing a recording — on continuously moving film — of the responses in the interarytenoid to such repetitive stimulation in this case no reflex discharge appeared until after the ninth stimulus As the number of stimuli increased the responses occurred more regularly their amplitude increased and at the same time the latency was shortened

Even *after* such a period of repetitive stimulation the amplitude of the reflex responses to a single stimulation of the internal laryngeal nerve was increased Fig 3 *D* shows a reflex response obtained from the interarytenoid muscle in the course of such a post tetanic facilitation For comparison see the weaker reflex response in Fig 3 *C* which was evoked before the facilitation the site of the recording electrodes is the same in both cases The response in the interarytenoid is now of about the same amplitude as the pretetanic responses in the cricothyroid (*A*) and the thyroarytenoid (*B*) muscles (recorded at the same amplification)



F g 8 Comparison of flex discharges in *A* and *B* cr cothyro d *C* and *D* thyroary teno d muscle evoked by timulation f ps lateral (*A* nd *C*) and ntralat ral (*B* and *D*) internal laryngeal nerv See text Latencies in *A* 10 ms *B* 12 msec *C* 13 msec *D* 2? msec Time b r 10 msec



F g 9 Simultaneous ec ding in poster or cricoaryten d (uppe beam) and in thyroaryt no d muscl (lower beam) of responses to psil t eral (*A*) and contralateral (*B*) timu l tion of internal laryngeal nerve See text T me b 20 msec

Contralateral reflex responses In a series of five experiments applying afferent stimulation to the contralateral internal laryngeal nerve the reflex responses in the adductors proved to be far less readily evoked than by ipsilateral stimula tion In the cricothyroid for instance the reflex discharge was very inconstant and when evoked (Fig 8 *B*) was of shorter duration and somewhat longer latency than the ipsilateral response (*A*) Although not shown by this example the amplitude of the contralaterally evoked responses was usually lower A silent period in the tonic activity similar to that shown in Fig 5 *B* could however be observed in most experiments Also in the thyroarytenoid the crossed responses did not appear regularly and when present (Fig 8 *D*) were of longer latency and lower amplitude than those elicited from the ipsilateral side (*C*) No crossed reflex responses were obtained by single contralateral stimuli in the lateral cricoarytenoid nor in the interarytenoid muscles but they could sometimes be evoked on repetitive stimulation Evidence of contralateral synaptic connections to motoneurons of these muscles was also obtained by the fact that bilateral simultaneous stimulation may elicit a feeble reflex response although either ipsi or contralateral stimulation alone had no effect



Fig 10 Responses to stimulation of intact nerve branch to *A* thyroarytenoid *B* cricothyroid muscle See text Time in *A* 10 msec in *B* 4 msec

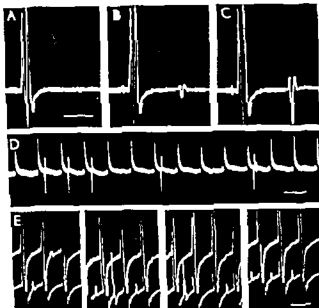
The response to contralateral stimulation elicited in the abductor muscle was an inhibition of the spontaneous activity which — contrary to the adductor responses — was equally pronounced as that to ipsilateral stimulation Fig 9 showing an experiment with simultaneous recording from the thyroarytenoid and the posterior cricoarytenoid muscles may serve as an illustration of the differences in efficiency of the contralateral reflex transmission to the abductor and the adductors respectively In this case the stimulus strength has been adapted so that the ipsilateral (*A*) and the crossed (*B*) reflex inhibition of the abductor at a constant level of the pre existent tonic activity are of about the same duration (upper beam) There is an obvious difference in amplitude between the ipsilateral and the crossed adductor responses at these stimulus strengths (lower beam)

2 Stimulation of the individual nerve branches to the intrinsic laryngeal muscles

In a series of experiments on some twenty dogs the individual nerve branches to the different intrinsic laryngeal muscles were stimulated in order to study whether proprioceptive reflexes could be obtained Most experiments were performed on the thyroarytenoid and cricothyroid muscles whose nerve branches could most readily be dissected free for application of the electrodes a few experiments were also done on the lateral and posterior cricoarytenoid muscles (cf Fig 1)

In some experiments both the stimulating and recording electrodes were placed at the central end of the cut muscle nerve but as a rule the stimulating electrodes were applied to an intact muscle nerve and the recording electrodes placed in the muscle The results obtained in this latter type of experiments will first be described

A typical recording from the thyroarytenoid in response to supramaximal single shock stimulation of the intact muscle nerve is shown in Fig *10 A* The first large muscle response which is due to the efferent nerve volley is followed by a second response of considerably lower amplitude after an interval of *17* msec Such secondary responses could also be evoked in the lateral and posterior cricoarytenoid muscles and were then of the same latencies as those recorded in the thyroarytenoid A corresponding recording from the cricothyroid at faster



F g 11 A—C pontaneous fluctu t n in l tency and ampl tud f sec d ry sponse n lat l cr aryt no d muscl Tim b 10 msec D interm ttent oc currence in posterio cr co aryteno d muscl of sec nd ary esponses (mall di phas sp kes) to f que t timulat n (20/se) Time 50 mse E independent variat ns of secondary ponses(mall upw d p kes or not hes) multa eously eco ded in tw d fferent parts of cri thyro d muscle T m bar 10 msec.

sweep speed is shown in Fig 10 *B* in this case the interval between the two responses is shorter 7 5 msec which is characteristic of this muscle An explanation for these differences in latency will be suggested below

In all muscles the secondary responses seemed to represent well synchronized motor unit discharges On repeated stimulations of constant strength (*e g* every two seconds) the secondary response exhibits pronounced variations both in regard to latency and amplitude Spontaneous fluctuations as large as those shown in Fig 11 *A—C* are common Characteristic of the secondary response is also that it drops out on certain stimuli in a series independently of the frequency of the stimulation a typical record showing this phenomenon at a frequency of 20/sec is shown in Fig 11 *D* Such a drop-out of the responses can also be seen in Fig 11 *E* illustrating secondary discharges elicited at a frequency of 100/sec and recorded simultaneously from two different sites in the muscle The figure also shows that the amplitude of the secondary responses varied in different parts of the muscle Under experimental conditions favoring the appearance of secondary discharges the average amplitude (calculated on about 100 successive responses) amounted to about 5 per cent of that of the primary responses judging from estimates from three experiments on the thyroarytenoid the lateral cricoarytenoid and the cricothyroid muscles In some instances the secondary responses only appeared in the shape of small notches on the base line (Fig 11 *E* upper beam) corresponding to only about one or two per cent of the primary response



Fig 12 A muscle responses to stimulation of intact nerve branch to cricothyroid muscle B same preparation after cutting of the nerve close to muscle stimulating and recording at central end of nerve Superimposed sweep pictures Time bar 4 msec

A closer analysis has been made of the secondary response in order to study its origin In view of the long interval between the two muscle responses the secondary response is *a priori* not likely to be a repetitive muscle response to the efferent nerve volley This alternative could actually be definitely excluded since the secondary response disappears altogether when the nerve is cut central to the stimulating electrode It could also be ruled out that the secondary responses were the result of axon reflexes since they dropped out after the vagus nerve had been crushed central to the nodose ganglion These observations must instead be considered as evidence indicating a central origin of the secondary response which also fits in with the observation that the latencies of these responses were longer in the muscles whose peripheral conduction distance was longer (cf Fig 1)

A centrally relayed response should of course be possible to record also from the motor nerve As a test the following procedure was applied in a small number of experiments After having recorded the secondary response in the muscle the muscle nerve was cut and stimulating and recording electrodes were placed on the central end of the nerve Fig 12 shows the results from such an experiment on the cricothyroid To the secondary response in the muscle recording (*A*) corresponds a small but distinct response of corresponding latency in the nerve recording (*B*) Like the muscle response the nerve response varied somewhat in latency and amplitude but had a lower average amplitude which may be explained by shunt effects in the recording from intact nerve In view of the technical difficulties involved in a recording from the laryngeal nerves the main analysis of the secondary response was performed on the basis of the muscle recording

When a central origin of the secondary response is thus established two alternatives have to be considered in view of corresponding analyses at the spinal level either it is a monosynaptic reflex response to stimulation of afferent fibers in the muscle nerve or a recurrent discharge from cells in the motor nucleus in response to the antidromic volley in the motor fibers The responses in the laryngeal muscles are however less readily analyzed than those obtained at the spinal level since as appears from the introduction no definite data are at hand about the afferent systems in the laryngeal muscles and their central connections Anyhow by performing an analysis in analogy with certain



Fig 13 Constancy of secondary response in lateral cricoarytenoid muscle at *A* submaximal and *B* maximal stimulus strength Time bar 10 msec

previous investigations of proprioceptive reflexes and recurrent discharges in the spinal cord it seems possible to arrive at a conclusion about the nature of the secondary responses

One way to approach this problem is to study the variations of the amplitude of the secondary responses at different stimulus strengths In experiments of this type the spontaneous variations in amplitude of these responses must of course be taken into consideration On the basis of a great number of experiments the following data were established The primary response always appeared at lower stimulus strength than the secondary independently of whether the cathode of the stimulating electrode was placed next to the muscle or not If the stimulus strength was further increased when both responses had been obtained the increase of the secondary response was approximately proportional to that of the primary response Deviations in either direction from this proportionality have been observed but as shown in Fig 13 equally strong responses could always be elicited at submaximal (*A*) as at maximal (*B*) stimulus strength

Judging from these data and from observations by MAGLADERY *et al* as per below the secondary response should not be a monosynaptic reflex response In human experiments using fundamentally the same experimental arrangement as in this work MAGLADERY *et al* (1951) found that the amplitude of monosynaptic reflex responses at first increased with rising stimulus strength but was reduced when the stimulus strength approached maximal values which was interpreted as an inhibitory effect of the antidromic volley (cf BROOKS *et al* 1950) Instead the secondary responses seem to resemble the recurrent discharges described by RENSHAW (1941) in experiments on the cat s ventral roots He observed that the size of such efferent discharges was roughly proportional to the size of the antidromic volley

The secondary responses were also studied while the experimental animals were put under a progressive depth of anesthesia by means of intravenous injections of repeated doses of barbiturates In these experiments infusion of thiogenal up to the stage of arrested respiration and abolished patellar reflexes was found not to cause any reduction of the secondary response. In some instances changes were recorded in the spike configuration which seemed to be a direct result of the administration of barbiturates

Recording of afferent muscle discharges According to available data in the literature monosynaptic reflex responses may occasionally be more sensitive to narcosis than are multisynaptic responses (BROOKS and KOIZUMI 1953) It could thus not be excluded that monosynaptic reflex connections to the laryngeal muscles are present although depressed and thus masked while multisynaptic reflex responses could readily be evoked In order to elucidate the question whether proprioceptive reflexes can be evoked from the laryngeal muscles it was considered to be of interest to study the presence of afferent systems in the muscle In a special series of experiments on eleven dogs attempts were therefore made to record afferent impulses from the separate muscle nerves *in toto* and after splitting them As a comparison the sternohyoid muscle was tested as to the presence of stretch receptors at the beginning of each experiment It proved to be comparatively easy to record afferent activity from this muscle and these preliminary experiments could thus serve as a control of the recording technique It could also be established that the afferent discharges derived from muscle spindles For this purpose the nerve was divided into two parts and one of them stimulated the discharges then ceased as long as the muscle was contracted

No afferent discharges were however obtained when the cricothyroid or the thyroarytenoid muscles were subjected to stretch induced either by dislocation of the cartilages on which the muscles have their attachments or by stimulation of an antagonist thus trying to imitate physiological stretch

At the present stage of the investigation it is thus not possible to demonstrate that the intrinsic laryngeal muscles in the dog contain any muscle spindles or anyhow that they are present in sufficient numbers to be of significance for the functional organization These experimental findings also seem to be confirmed by preliminary results obtained in a recently started series of histological investigations (MÅRTENSSON and WIRSEN unpublished work)

Data have however been published as to the presence of other end-organs than muscle spindles which have been interpreted as proprioceptive (*e g* SUNDER PLASSMAN 1933 RUDOLPH 1961) and which might be thought to be innervated by fibers of considerably smaller diameter than afferent fibers from muscle spindles With the experimental technique used in the present investigation it is quite possible that an afferent system of that type although present may not have been detected (cf Discussion) Further investigations are thus necessary before it is possible definitely to solve the problem whether a proprioceptive system is present in the intrinsic laryngeal muscles

Discussion

The function of the various laryngeal muscles participating in the reflex closure of the glottis has previously been analyzed in dogs by YAMASHITA and URABE (1959 1960) who found that stimulation of the internal laryngeal nerve caused reflex discharges not only in the two adductors studied *m* the crico-

thyroid and the lateral cricoarytenoid but also in the abductor the posterior cricoarytenoid However in the present investigation reflex discharges were never observed in the abductor only inhibition of pre existent activity was recorded concomitantly with the activation of the adductors Nor do YAMASHITA s and URABE s results fit in with the classical concept of the abductor and the adductors as being antagonists and their interpretation of the results obtained does not seem convincing It is of course possible that under certain experimental conditions the synchronous afferent volleys produced by electrical stimulation may open up reflex connections normally closed (cf KOLMODIN 1957) and evoke a reflex response in the abductor but there is also the possibility that the results obtained by YAMASHITA and URABE are due to insufficiently selective recording conditions resulting in a pick up of activity from some nearby muscle

In electromyographic studies of the activity of the intrinsic laryngeal muscles in man FAABORG ANDERSEN (1957) found that an increase of the tonic activity in the adductors and an inhibition of the abductor activity occurred with cough and deglutition which corresponds to a reciprocal innervation pattern

In studies of deglutition movements reflexly evoked by repetitive electrical stimulation of the superior laryngeal nerve in monkeys cats and dogs DOTY and BOSMA (1956) showed that also in these species certain laryngeal muscles are engaged in the swallowing process The repetitive stimulation led to prolonged discharges in the two adductors studied the thyroarytenoid and cricothyroid muscles and besides in response to individual stimuli brief reflex discharges could be recorded which were of the same type as those observed in the present experiments and also showed similar facilitation phenomena

In the present study a silent period in the tonic activity was observed following the reflex discharges in the adductors (cf also DOTY and BOSMA 1956) In the cricothyroid cessation of pre existent activity was sometimes observed even without any more distinct previous reflex discharge an effect which resembles that recorded in the posterior cricoarytenoid Whether different central mechanisms are behind the inhibitory phenomena in the two muscles can only be decided by means of recording from the central nervous system but it might be worthwhile to discuss different alternatives The reciprocal inhibition in the posterior cricoarytenoid is most likely due to a synaptic inhibitory process initiated concomitantly with the adductor activation in analogy with the mechanisms for reciprocal inhibition in the spinal cord The silent period observed in the cricothyroid and other adductors may also be due to a similar synaptic inhibitory process but another obvious alternative is a postexcitatory subnormality either in the motoneurons proper or in an interneuron chain

The repetitive discharge type of the laryngeal reflex responses supports the concept that these reflexes are multisynaptic like other protective reflexes for instance warding-off reflexes on cutaneous stimulation this view is also sup-

ported by approximate calculations of the central reflex time The exact nature of the adductor reflexes can however only be determined by further exploration with intracranial electrodes

Of the reflex responses obtained on single shock stimulation of the adductors those recorded in the lateral cricoarytenoid and the interarytenoid muscles are generally of lower amplitude than those in the thyroarytenoid and the cricothyroid This may be due to physiological differences in the transmitter potentiality of the reflex connections However the concept of a limited reflex transmission to the lateral and interarytenoid muscles under normal physiological conditions seems inconsistent with the fact that repetitive stimulation may result in a facilitation of the low amplitude responses in these muscles Besides it would no doubt be inadequate if the level of reflex activation should be considerably lower in the interarytenoid than in the thyroarytenoid muscle, since the glottic closure would then be ineffective the posterior part of the glottis being normally closed by the interarytenoid muscles drawing the arytenoid cartilages close to each other

It is therefore possible that the differences in amplitude of the various ipsilateral reflexes are due to the special experimental conditions, and this may apply also to the differences observed between contra and ipsilateral reflex transmission The low amplitude responses were generally of longer latencies and it has previously been shown that reflexes of a longer central delay are more readily inhibited by barbiturates than those of shorter delay (PETERSÉN 1952) It can thus not be entirely excluded that the differences in reflex transmission effectiveness may be due to a selective sensitivity to the narcosis

It seems justified to presume that under natural physiological conditions the crossed reflex transmission to the adductors is more effective than it has proved to be in experiments using single shock stimulation On natural stimulation of nerve endings in the mucosa there is an afferent inflow of repetitive impulses and in the present experiments repetitive volleys have been shown to facilitate both contra and ipsilateral reflex responses A bilateral adduction of the vocal cords in response to stimulation of any side of the laryngeal mucosa also seems to represent the most appropriate reflex system for the adequate protection of the respiratory tract

Also from a clinical point of view studies of the reflex effects of the laryngeal mucosa afferents may be of some interest Thus for instance the laryngospasm provoked by a foreign body in the glottic aperture or by ether narcosis may be due to an increased impulse flow in the mucosa afferents induced by the excitatory effect on the mucosa and resulting in an abnormally strong adductor tonus This may also account for the clinical experience that the laryngospasm under narcosis may be prevented by surface anesthesia of the mucosa since by this procedure the afferent impulse flow is reduced

In attempts to record afferent impulses from the intrinsic laryngeal muscles no results have been obtained suggesting the presence of muscle spindles It is

however impossible to say whether a proprioceptive system is entirely absent since in histological studies of these muscles particularly in homo other types of receptors have been described to which proprioceptive functions have been attributed (*e g* SUNDER PLASSMAN 1933 RUDOLPH 1961) and such receptors may be present also in the dog For various reasons it is possible that impulses from such receptors may not be revealed by the recording and stimulating technique used in this investigation in the first place muscle stretch may perhaps not be the adequate stimulus type and besides these receptors may be innervated by small nerve filaments which are easily injured In cat experiments BIANCONI and MOLINARI (1960 1961) have recorded afferent activity in the recurrent laryngeal nerve which has been interpreted as proprioceptive impulses from different laryngeal muscles but it is hardly possible to draw definite conclusions from results obtained in the way described in their preliminary reports *viz* by recording from the whole recurrent nerve

At the present stage it is thus not possible to establish whether the laryngeal muscle nerves in the dog are composed of pure motor fibers or if they contain also proprioceptive fibers In the interpretation of the responses to stimulation of the muscle nerves a central interaction between an antidromic and a possible proprioceptive volley has to be taken into account the nature of the secondary responses is thus a very intricate problem Some aspects will however be discussed below in an attempt to elucidate the question by pointing out the differences and similarities existing between the secondary responses on the one hand and the monosynaptic reflex responses and the recurrent discharges on the other hand

It is known that monosynaptic reflex responses elicited by stimulation of muscle nerves are reduced in amplitude as the stimulus strength approaches maximal values (MAGLADERY *et al* 1951) this is a consequence of an increasing antidromic inhibition of the motoneurons (BROOKS *et al* 1950) In the present experiments however the amplitude of the secondary responses increased fairly proportionally to the increase of the stimulus strength They resembled in this respect recurrent discharges recorded from the cat s ventral roots (RENSHAW 1941) Further characteristics of the secondary responses will be described below which favor the view that these responses correspond to this type of efferent discharges

Recurrent discharges have previously been recorded from the cat s spinal roots where they amounted to 2—3 per cent of the antidromically activated fibers (RENSHAW 1941) and the secondary responses recorded in the laryngeal muscles were of about the same size It seems as if recurrent discharges at the spinal level show only small variations in amplitude (cf BROOKS *et al* 1950) the secondary responses recorded in the present experiments showed pronounced variations in amplitude and were not regularly obtained on each stimulation. However the recording conditions for these phenomena are different in spinal ventral roots and in the laryngeal muscles According to hypotheses advanced

(BROOKS *et al* 1950 ECCLES 1955) recurrent discharges may be initiated if an antidromic impulse invades the soma after a long delay at axon hillock. It has also been shown that the presence of such a delay is dependent upon the polarization level of the motoneuron (BROCK *et al* 1953 COOMBS *et al* 1955) It might be suggested that the motoneurons of the laryngeal muscles should exhibit more pronounced spontaneous variations in excitability level than the spinal motoneurons because they are engaged in the respiratory activity (cf ANDREW 1955 GREEN and NEIL 1955 FAABORG ANDERSEN 19.7) If the concomitant changes in polarization level of the laryngeal motoneurons are presumed to influence the initiation of recurrent discharges in the same way as in spinal motoneurons it is evident that the motoneurons of the laryngeal muscles are more liable to variations in recurrent discharges than are the spinal motoneurons The phenomenon has so far mainly been studied by means o needle electrodes in the laryngeal muscles which implies a selected variety o motor units and this may also have contributed to the greater variability observed in the discharges as compared with the responses obtained on ventral root recording

It has also been shown that barbiturates cause depression of antidromic *axon soma transmission (cf BROOKS and ECCLES 1947) and these may* favor the occurrence of a long delay in transmission at the axon soma junction and a recurrent discharge This may explain why it has been possible in these experiments to elicit secondary responses of the same amplitude in deep as in superficial narcosis which would not have been expected if the secondary response had represented a monosynaptic reflex response (cf BROOKS and ECCLES 1947 PETERSÉN 1952) It should also be mentioned in this connection that BROOKS *et al* (1950) have reported that recurrent discharges in spinal nerves occur more readily in anesthetized than in decerebrate animals

It is likely that the human phonation mechanism requires a finer coordination of the intrinsic laryngeal muscles than is necessary in other species It cannot therefore be excluded that the human laryngeal muscles may contain a well developed proprioceptive system and a large number of muscle spindles have also been claimed to exist in these muscles (LUCAS KEENE 1961 KÖNIG and LEDEN 1961) In human operations ESSLEN and SCHLOSSHAUER (1960) have studied the problem experimentally by stimulating the intact nerves to the larynx and recording reflexly evoked responses from different laryngeal muscles They interpreted the reflex responses as monosynaptic and asserted that the proprioceptive impulses travel mainly through the superior laryngeal nerve and only to some minor extent through the recurrent nerve *However* they applied stimulation to mixed nerves containing both interoceptive afferents and muscle nerves and their interpretation of the responses obtained is therefore somewhat uncertain there is so far no experimental support for the positive histological findings

Summary

The present work is the first in a series of investigations — in the dog — of eflex mechanisms in the larynx The purpose of this paper has been (1) to tudy the reflex responses obtained in different laryngeal muscles on stimulation if the internal laryngeal nerve with a view to elucidating the mechanism of the protective reflex closure of the glottis (2) to analyze the responses obtained on stimulation of separate muscle nerves for the purpose of studying whether the aryngeal musculature contains a proprioceptive system

On single shock stimulation of the ipsilateral internal laryngeal nerve, reflex discharges of an asynchronous type and of long central delay were observed in all the adductors studied the discharges have been interpreted as being multisynaptic reflex responses If a reflex discharge was elicited during a phase of pre existent tonic activity it was followed by a silent period such a period might also occur without a preceding distinct reflex discharge In the lateral cricoarytenoid and the interarytenoid muscles the responses were less readily evoked and of lower amplitude than those obtained in the cricothyroid and thyroarytenoid muscles

Stimulation of the ipsilateral internal laryngeal nerve did never elicit a reflex discharge in the abductor muscle the posterior cricoarytenoid but regularly caused inhibition of pre existent tonic activity in this muscle the duration of the inhibition varied with the stimulus strength and with the intensity of the pre existent background activity

On single shock stimulation of the contralateral internal laryngeal nerve reflex discharges could be recorded only in the cricothyroid and the thyroarytenoid muscles they were of shorter duration and/or of lower amplitude and of longer latency than those obtained on ipsilateral stimulation Conclusive proof of latent contralateral reflex connections to the lateral cricoarytenoid and interarytenoid muscles was however obtained since *e g* contralateral stimulation could be shown to facilitate ipsilateral reflex responses

Repetitive contralateral — as well as ipsilateral — stimulation facilitated the reflex responses in the adductors and discharges could also be evoked in muscles not activated by single afferent stimuli post tetanic facilitation was also demonstrated

On stimulation of the contralateral internal laryngeal nerve no reflex discharges were elicited in the abductor the posterior cricoarytenoid but only an inhibition of the pre existent tonic activity which was equally pronounced as on ipsilateral stimulation

The functional significance of the reciprocal innervation pattern for the adductors and the abductor thus demonstrated is discussed

Stimulation of intact muscle nerve branches evoked besides a direct response from the muscle a secondary response amounting to a few per cent of the

primary response. The synchronous secondary response varied considerably in amplitude and latency and appeared intermittently on repetitive stimulation. In view of the characteristic behaviour of this response, i. e. during variations in the stimulus strength and in the depth of the narcosis, the secondary response was interpreted as being a recurrent discharge from the motoneurons, whereas no response exhibiting the properties of a monosynaptic reflex was observed under the prevailing experimental conditions.

In recordings from motor nerve filaments from different intrinsic laryngeal muscles subjected to stretch no impulses from muscle spindles could be recorded; the presence of muscle afferent systems of other types, e. g. with nerve fibers of fine calibers, can however not be excluded.

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Studies on the Relationship Between Flow Resistance, Capillary Filtration Coefficient and Regional Blood Volume in the Intestine of the Cat

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Abstract

FOLKOW B O LUNDGREN and I WALLENTIN *Studies on the relationship between flow resistance capillary filtration coefficient and regional blood volume in the intestine of the cat* Acta physiol scand 1963 57 270—283 — The relation between flow resistance regional blood volume and capillary filtration coefficient (CFC) in the cat's intestine has been quantitatively studied at different levels of vascular smooth muscle activity and compared to the vascular bed of the skeletal muscles The resistance vessels of booth these vascular circuits maintain a considerable basal tone but the intestine is far richer vascularized which is obvious from the figures given for flow resistance regional blood volume and CFC at comparable levels of vascular tone including maximal vasodilatation Intestinal CFC is both in absolute and relative terms higher than in the skeletal muscles as reflected by a definitely larger CFC/blood flow ratio over the entire range of vascular tone CFC thus increases considerably when vascular tone is reduced and reaches very high figures at maximal asodilatation This is thought to reflect an opening up of additional capillaries due to relaxation of their precapillary sphincters Reasons are given for the belief that the glandular mucosal portion is far better vascularized than the smooth muscle portion of the intestine and at maximal vasodilatation CFC of the mucosal portion may be of the order of 1 ml/min Hg/mm/100 g of tissue Such a figure is in fact comparable to the renal glomerular filtration when expressed in the same way

For several years the different parallel coupled circuits of the systemic vascular bed have been studied from a quantitative point of view in this laboratory Obviously these circuits are designed to suit the need of their particular tissues and can for such reasons be expected to show considerable differences

between their functionally specialized series-coupled sections with respect to both dimensional characteristics and smooth muscle control (FOLKOW 1959). To start with, the control of the resistance vessels within different tissues as exercised by nervous, local chemical and myogenic factors was investigated (e.g. FOLKOW 1955, 1960). More recently these studies aiming at more quantitative information of integrated vascular control have been extended to include also the other functionally different sections of the individual circuits. First the vascular circuits of the skeletal muscle and the skin were studied from such a point of view and a technique was developed which allows a continuous and quantitative recording of events taking place in the different consecutive vascular sections under fairly physiological conditions (FOLKOW 1959, MELLANDER 1960, FOLKOW *et al.* 1961, FOLKOW and ÖBERG 1961, COBBOLD *et al.* 1963).

The present series of experiments was begun in order to explore the intestinal vascular bed in a similar way. Secretion and absorption — dominant features in intestinal function — no doubt require a vascular bed that in several respects must differ considerably from that of e.g. the skeletal muscle or the skin. Characteristics such as the extent of resting blood flow as related to maximal flow capacity, the arrangement of the capillary section and its control via precapillary sphincters, the regional blood volume and its variations brought about by changes in the tone of the capacitance section are of interest here in addition to the organization of the superimposed nervous and local chemical control mechanisms. This first study of the intestinal vascular bed has concentrated on an exploration of the relationship between blood flow, regional blood volume and capillary filtration coefficient (CFC) at different levels of vascular smooth muscle tone. These general characteristics were thought to be of some interest, the more so as the same parameters have recently been studied in some detail with respect to the vessels of the skeletal muscle (MELLANDER 1960, COBBOLD *et al.* 1963). A brief survey comparing the abovementioned characteristics of the intestinal and muscle vascular circuits has been given by COBBOLD *et al.* (1962). In subsequent papers factors such as the range of control exercised by the vasoconstrictor fibres within the different consecutive vascular sections will be discussed. A preliminary report concerning these latter aspects has been given by LEWIS, FOLKOW and MELLANDER (1962).

Method

A. Operative procedure

Experiments were performed on 20 cats weighing 2—3 kg, anesthetized intravenously with chloralose (50 mg/kg) and urethane (100 mg/kg) after induction with ether.

After insertion of a tracheal cannula, the abdomen was opened in the midline and the greater omentum and the spleen were extirpated. In many experiments a total gastrectomy was performed without disturbing the arterial blood supply to the liver to allow free access to the cognate vessels and nerves of the intestine and to make room for an intestinal plethysmograph. A section of the jejunum, usually weighing about 15 g

was chosen for the experiment and the remainder of the intestinal tract was extirpated During this procedure great care was taken to leave the cognate vessels and the nerves intact as well as the arcuate anastomosis between the inferior and the superior mesenteric arteries as the peripheral end of the divided inferior mesenteric artery was later used for close intraarterial infusions or injections into the intestinal segment to be studied In most of the experiments the effects of vasodilator and vasoconstrictor substances on the intestine were studied under circumstances in wich reflex nervous influences on the intestinal vessels and the intestinal wall were minimized in order to reveal the extent of the locally induced vascular eff cts For these experiments the right adrenal gland was therefore extirpated and the left one denervated The superior mesenteric artery and vein were completely stripped of the sourrounding nerve plexuses

The cat was then heparinized and the blood pressure was recorded from the right femoral artery by means of a mercury manometer The arterial inflow pressure to the intestine could be maintained at any desired level by adjustment of a screw clamp placed around the aorta at the level of the diaphragm The superior mesenteric vein draining the intestinal segment and its lymphatic glands was cannulated and connected to an optical drop recorder unit filled with inert silicone oil through which the drops of blood fell The drop recorder operated an ordinate writer in which the height of the ordinates was inversely proportional to the rate of flow The venous outflow pressure could be set at any desired level by adjusting the height of the tube which drained the drop recorder The venous outflow was returned to the animal via the right external jugular ve n The drop recorder unit and outflow tube system could also be kept entirely closed and the venous outflow pressure could then be changed by adjusting a clamp placed around the tube draining the drop recorder The exact value of the venous outflow pressure was always recorded by means of a water manometer coupled to the connection betv een the superior mesenteric vein and the drop recorder In this way both the arterial inflow and the venous outflow pressures were und r consta it control

To allow a continuous and sensitive record ng of changes in tissue volume the intestinal segment was enclosed in a triangular perspex plethysmograph The mesenteric root with the superior mesenteric artery and th cannulated mesenteric vein came out of the plethysmograph through a closely fitting opening at the proximal angle This opening was sealed with plastibase (SQUIBB) The wide cannula inserted into the mesenteric vein was always introduced far enough distally into the ein so that it was well inside this sealed opening thereby avo d ng collapse of the ein by undue lateral pressure With this arrangement the intestine as enclosed in the plethysmograph in a perfectly air and watertight way without interference with either the arterial inflow or the venous outflow and the motility and colour of the intestine could be d rectly inspected The plethysmograph was filled with Tyrode s solution at 38 C leaving only a small air volume in the upper outlet angle of the plethysmograph which was connected to a sensit ve and well trimmed volume piston recorder Th temperature inside the plethysmograph was continuously monitored with a thermocouple device and the contents of the plethysmograph maintained at 38 C with the aid of an nfrared lamp The temperature of the animal was also maintai ied constant at 37—38 C v ith a heating pad and an infrared lamp Drying and cooling of the exposed parts of the abdominal cavity was avoided by a plastic sheet

In some experiments the animal was artificially ventilated at a frequency and tidal volume wh ch closely simulated the normal respiratory events in order to el minat interfering effects of spontaneous changes in resp ration and blood composition during the course of the experiment.

Close intraarterial injections or nfusions of vasoactive drugs to the intest ne could be performed by means of a thin polyethylene catheter inserted into the peripheral end of

the inferior mesenteric artery which via the preserved arcuate connection made direct contact with the superior mesenteric artery As a vasoconstrictor drug l noradrenaline was used To produce intestinal vasodilatation isopropyl noradrenaline (Pafadrin PAF' AB Hassle) was utilized as this drug relaxes the intestinal smooth muscles also In th s way mechanical interferences vith the vasodilator respcnse could be avoided which is hardly possible when vasodilator substances like acetylcholine histamine etc are used These vasodilator drugs produce a contract on of the intestinal smooth muscles also which can markedly obstruct intestinal flow masking th extent of the concomitantly nduced vasodilatation

The blood volume contained within the acutely denervated intestinal segment and its mesenteric root in the steady state was n most cases determined at the end of the experiment procedures in the following way A sample of venous blood was drawn from the cat while at the same time the cognate artery and vein to the intestinal segment were clamped just at the ntrance to the plethysmograph The intestinal blood content v as then washed out with a kno vn amount of dextrane Tyrode s solution and the hematocrit and/or haemoglobin content of th s sample as well as of th venous blood sample v ere determined With the assumption that the hematocrit of the intestinal blood approxi mately equalled that of the venous blood the blood volume of the intestinal segment could be deduced from the extent of dilution caused by the known amount of dextrane Tyrode s solut on This can be expected to underestimate slightly the blood volume con ta ned in the intestin as the hematocrit n the small vessels should be somewhat lower th n in venous blood

B D termination of cap llary filtrat on fficient

After enclosing the intestin in the plethysmograph it was always poss ble to adjust the enous outflow pressure so that an isovolum tric state was attained implying that outward and inward filtration at the c pillary level equalled each other Thus isovolu metric equilibrium could then be d liberately d sturbed by increas ng temporarily the venous outflow pressure to a known extent This procedure evoked a cha a terist c olume increase in two phases which showed completely d fferent slope chara terist cs As earlier demonstrat d (see *e g* MELLANDER 1960) th first ra d change n volume is caused by vascular distent on ma nly of th thin walled veins v hile the continuous and slow r increase of the volume curve the filtr t on slope refle ts the outward filtration aused by th ra sed cap llary hydr tat c p essure

To be able to calculate the cap llary filt tion coefficient (CFC) t is ne essary to corr late th rate of outward filtrat on with the nduced hange n mean pillary hydrostat c p essure It annot however s mply be assumed that th applied venous pressure quant t tively t ansmitted n a retrograd fashion to the capillary level The ext nt to which this in as of venous pressure is propagated to the capillary level depends on th relat on betw en the p e and postcap llary es stance of the vascular bed This relat on between p e and postcap llary esistances could easily ha e been lculated provided that mean capillary pressure had been exactly known The method ut lized by PAPPENHEIMER t al (ee PAPPENHEIMER 1953) — changing both arte al and venous pressure to a known ext nt while measuring the effects of these pressure changes on capillary filt tion exchange — can unfortunately not be used n prepara t ons n which the vessels normally exhibit autoregulation as is the case with the ntes tinal vascular ci cu t Shifts n arterial pressure will then lead to concomitant ad ptive changes in tone confined essentially to the precapillary resistance vess ls (FOLKOW and ÖBERG 1961) thereby changing what was to be measured For such reasons Papp nhe mers m thod of estimating the mean capillary p essure can be uccessfully utilized only under circumstances in wh ch the precapillary resistance remains largely uninfluenced by

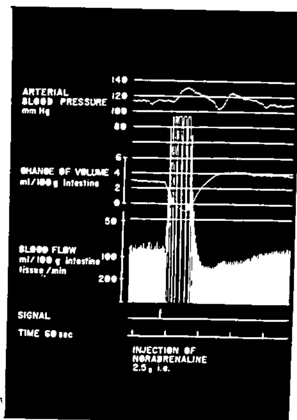


Fig 1 Effects of injection of noradrenaline on blood pressure intestinal blood flow and tissue vol m

changes in arterial pressure and flow Another indirect approach had to be used here. Intestinal lymph appears to contain as much as half the protein amount of the blood plasma (SWANN *et al* 1961) and therefore it can be expected that the colloid osmotic pressure gradient across the capillary walls is fairly low in this tissue Intestinal tissue pressure is not known but for good reasons it can be expected to be virtually negligible under normal circumstances In the isovolumetric state when filtration equilibrium is present it is therefore just fied to assume that mean capillary pressure is fairly low in the intestine and figure of 15 mm Hg has been considered to be reasonable here Thus at a mean capillary pressure of 15 mm Hg an arterial pressure of 100 mm Hg and a venous outflow pressure close to zero some 85 of an applied venous pressure rise will be transmitted to the capillary level i e 4 25 mm Hg when venous outflow pressure is raised 5 mm Hg In fact should the mean capillary pressure in reality be as high as 25 mm Hg or as low as 5 mm Hg — a priori not very likely — the error in the deduction of the pressure transmitted to the capillary level is nevertheless quite small To exemplify in the first instance it would be 75 % of 5 or 3 75 mm Hg and in the latter case 95 of 5 or 4 75 mm Hg instead of the 4 25 mm Hg pressure increase obtained if mean capillary pressure is 15 mm Hg as assumed above The consequent error in the calculation of CFC would for these wide limits set for mean capillary pressure only be some 10—15 wh ch is of minor importance for the present problem. It therefore seemed justified to base the calculation of intestinal CFC on a mean capillary

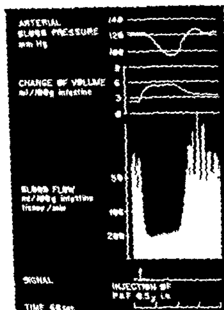


Fig 2 Effects of injection of isopropyl noradrenaline on blood pressure intestinal blood flow and tissue volume

pressure set at 15 *mm Hg* in the isovolumetric equilibrium By using such approximations CFC of the acutely denervated intestine has been calculated in the present experiments and related to the prevailing blood flow resistance and regional blood volume Furthermore despite wide changes in vascular tone it has been shown that there was relatively little effect on mean capillary hydrostatic pressure as judged from the volume of the intestinal segment Therefore while the absolute value for mean capillary pressure may be in error necessitating as mentioned a minor correction in resting CFC whatever capillary pressure is chosen the observations reflect the true extent of the change in CFC associated with the induced changes in vascular tone

Results

Fig 1 illustrates the shifts in blood flow and intestinal volume reflecting changes in blood content and tissue fluid as caused by a large dose of intra arterially injected noradrenaline At the peak of the noradrenaline effect flow resistance increased about 10—20 times Corresponding in time with this drastic increase of flow resistance tissue volume decreased some 3 per cent and the intestine was seen to blanch obviously due to expulsion of a considerable fraction of its blood content when the capacitance vessels constricted The extent of these vascular responses is approximately comparable to the peak effects obtained when all the sympathetic constrictor fibres to the intestinal blood vessels are stimulated at maximal rates (CELANDER 1959 FOLKOW *et al* to be publ) In most of our estimations of the blood volume within the acutely denervated intestine when measured in the isovolumetric steady state values around 7—9 ml of blood/100 g of tissue were obtained and the resting blood

flow was generally around 40—60 ml/min/100 g in the actual experiment it was somewhat higher When these values for resting blood content are related to the volume decrease caused by the noradrenaline injection in Fig 1 it is clear that some 30—40 % of the blood content was expelled *This volume decrease reflects a truly active constriction of the capacitance vessels and is only to a minor part due to a passive-elastic recoil* when the postcapillary transmural pressure is decreased by the intense precapillary resistance increase This is clear from the fact that even with total occlusion of the superior mesenteric artery when the transmuaral pressure of the capacitance vessels must be even more lowered tissue volume was far less decreased under the present experimental conditions

Fig 2 illustrates the changes of the same parameters in another experiment elicited by intraarterial injection of isopropyl noradrenaline (PAF) At a pressure head maintained around 100 mm Hg blood flow increased from some 50 to about 200 ml/min/100 g tissue but even so the resistance vessels were not maximally dilated This is clear from the fact that in most experiments (see also the diagram in Fig 4) still higher doses of isopropyl noradrenaline, or a period of complete ischaemia could increase flow to some 250—275 ml when the pressure head was maintained at 100 mm Hg Corresponding to the maximal increases of blood flow tissue volume generally increased maximally some 30—40 % obviously due to a concomitant relaxation and distension of the capacitance vessels

The potential range of control of the intestinal vascular bed as normally exercised by the centrally controlled constrictor fibres on the one hand and by locally produced vasodilator factors on the other may thus be very wide Expressed in terms of the ratio of the pressure head in mm Hg and the flow in ml/min/100 g of tissue (peripheral resistance units PRU) the flow resistance within the cat s intestine was under the prevailing resting conditions about 1 5—2 5 PRU It could be increased to around 30 PRU at constrictor responses which correspond in extent to the peak effects produced by maximal constrictor fibre activation It could be reduced to about 0 4 PRU when the vascular smooth muscles were maximally relaxed Resting regional blood volume (approximately 7—9 ml/100 g) may be expected to be decreased by some 30—40 % at intense sympathetic discharge and increased to about the same extent by local vasodilator factors

Fig 3 illustrates the effects on tissue volume and blood flow caused by a sudden venous pressure rise An immediate and moderate increase in tissue volume is obtained while at the same time venous outflow is temporarily reduced This rapid increase of tissue volume and phasic flow decrease are due to pooling of blood within the distended capacitance vessels when they are exposed to the increased transmural pressure This initial phase of volume increase is followed by a second much slower but quite steady phase This second continuous volume increase reflects an outward capillary filtration as

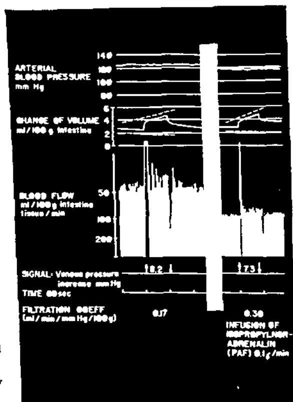


Fig 3 Effect of venous pressure rise on intestinal volume and blood flow before and during infusion of isopropyl noradrenaline Note the increase of blood flow and calculated CFC during the infusion of isopropyl noradrenalin

has been demonstrated in several studies utilizing different techniques (*e g* MELLANDER 1960) It thus allows a deduction of CFC if the mean capillary pressure rise can be evaluated It has earlier been stated that in the isovolumetric state mean capillary pressure is assumed to be of the order of 15 mm Hg

If this assumption is correct it implies that some 85 of a venous pressure rise is transmitted to the capillary level when arterial inflow pressure is 100 mm Hg and venous outflow pressure close to zero It has also been noted that this quotient is only little changed if mean capillary pressure should be as high as 25 or as low as 5 mm Hg instead of 15 In this way CFC can be calculated with only a fairly small error as reasonably correct values for volume increase per unit time total tissue weight and mean capillary pressure rise are available — In the left section of the experiment shown in Fig 3 blood flow through the acutely denervated intestine was about 50 ml/min/100 g and CFC was deduced to be 0 17 (ml/min/100 g/mm Hg) In the right hand part of the figure a moderate but steady vasodilatation was induced by an intraarterial infusion of isopropyl noradrenaline which increased the blood flow level to 90 and CFC to 0 30

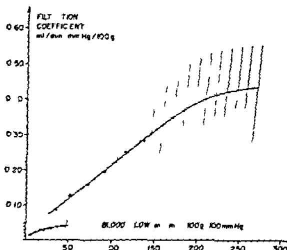


Fig 4 Cumulated data on the correlation between intestinal blood flow and CFC, where graded vasodilatations have been induced by means of infusions of isopropyl noradrenalin. Dots indicate means of classed values, hatched area represents approximate range. As a comparison the correlation between blood flow and CFC in the skeletal muscles (from Cobbold et al. 1963) is inserted also (curve in left lower corner of the diagram)

CFC has been repeatedly estimated in a total of 20 experiments in which different degrees of vasodilatation up to the maximal level were induced by steady intraarterial infusions of isopropyl noradrenaline in various concentrations. The correlation between the prevailing blood flow — i. e. the extent of tone of the resistance vessels — and CFC is shown in Fig 4. The curve based on classed mean values with an indication of the spread among the individual estimations in the form of the hatched area represents altogether 123 individual measurements of CFC. As a comparison the relationship between blood flow and CFC for skeletal muscle is also inserted in the same diagram, the latter data taken from Cobbold et al (1963). In both instances there are good reasons for assuming that the CFC increase accompanying the flow increase which was produced by a vasodilator drug in the intestine and by increased tissue activity in the skeletal muscle is caused by opening up of previously closed capillaries. This can be expected to occur when their smooth muscle sphincters become relaxed, which then makes the capillaries available to the blood flow and hence for filtration exchange. — In a minor group of the experiments the intestinal vessels were kept in a fairly steady state of increased tone by intraarterial infusion of noradrenaline. It was then generally found that CFC decreased roughly in proportion to the increase of flow resistance; these few measurements are not included in the diagram of Fig 4. Fig 4 also illustrates the wellknown fact that the intestine is far better vascularized than skeletal muscle, the resting blood flow level of the former tissue being as big or even bigger than the maximal blood flow capacity of the latter. At these equally big blood flow levels per unit weight of the two tissues it is further worth noting that the intestinal CFC is almost three times bigger than that of the skeletal muscle. In fact this difference in CFC/blood flow ratio which is present at maximal intestinal vasodilatation also is probably in reality even

bigger for the following reasons The intestinal segment studied generally included a group of lymph glands surrounding the main vessels and amounting to some 25—35 % of the tissue volume studied In some of the experiments blood flow and CFC of these glands were measured separately after extirpation of the intestinal segment Generally their resting blood flow was around 30—60 ml/min/100 g while CFC was then only some 0 02—0 04 (ml/min/100 g/mm Hg) This CFC/blood flow ratio for the lymph glands corresponds more closely to that of skeletal muscle than to intestine When the blood flow and CFC figures for the intestine proper are corrected for this admixture of lymph gland tissue intestinal CFC for a given blood flow level is in fact somewhat higher than that indicated in Fig 3 and 4

Discussion

A considerable number of earlier investigations (for lit see *e g* LACROIX 1960 and GRIM 1962) have dealt with the regulation of the intestinal vessels and though most of them have been confined to estimations of blood flow resistance aspects such as regional blood volume capillary exchange etc. have sometimes been considered also However only more recently have methods been developed by which several of these parameters have not only been measured concomitantly but also to some extent quantitatively analyzed For instance JOHNSON (1959 1960) has adapted PAPPENHEIMER and SOTO-RIVERA s gravimetric method (1948) originally designed for studies of the capillary exchange in the hind limb for a thorough study of blood flow autoregulation in the dog s intestine As this technique like the present variant of Pappenheimer and Soto-Rivera s method allows a concomitant recording of changes in blood volume and CFC it offers possibilities for detailed studies of the intestinal vascular events With an entirely different approach using labeled red cells and albumin SWANN *et al* (1961) have analyzed in more detail some aspects of the exchange between the intestinal vascular bed interstitial space and lymphatic system A far better insight into the complex control of the gastro-intestinal vessels can be expected to be obtained with the aid of such methods

It is also wellknown that the intestine is richly vascularized having a big blood supply a remarkably dense capillary network and a fairly big blood content The present findings are in complete agreement with these general views and may offer more detailed information concerning these parameters To start with the range of the smooth muscle control of the intestinal resistance vessels will be discussed The present experiments were deliberately performed on intestinal segments which were acutely deprived of both their vagal innervation and vasoconstrictor fibres It was incidentally observed that the parasympathetic denervation of the intestinal structures did not significantly affect the circulatory events under the present circumstances presumably because there was no significant activity of these centrally directed nerve fibres control

ling motor and secretory events Elimination of the tonically active vasoconstrictor fibres on the other hand naturally increased blood flow and tissue volume somewhat leaving them in the state of basal tone determined by local mechanisms alone Aspects of the range of control, exercised by the vasoconstrictor fibres will be described in another study (FOLKOW et al 1962) Under the circumstances of the present study corresponding to a situation of reflexely inhibited sympathetic activity in the intact organism, the resting intestinal blood flow was generally of the order of 40—60 ml/min/100 g at a pressure head around 100 mm Hg At maximal relaxation of the intestinal blood vessels flow was seen to increase up to some 250—275 ml The corresponding figures for skeletal muscles is in our experience 6—10 ml and 40—50 ml/min/100 g of tissue respectively The big difference between resting and maximal blood flow shows that both vascular circuits maintain a considerable basal tone of their resistance vessels (LOFVING and MELLANDER 1956) and it is wellknown that both exhibit autoregulation of blood flow (e g FOLKOW 1949 JOHNSON 1959 1960 TEXTER et al 1962) as is the case with several other circuits This phenomenon like the basal tone seems in the final analysis to be an expression of a truly myogenic activity of the resistance vessels (FOLKOW 1962) though it normally is modified to a considerable extent by extrinsic factors

The huge intestinal blood supply both at rest and especially at maximal dilatation calls for some remarks The heterogenous intestinal tissue is grossly speaking made up of a smooth muscle portion and glandular mucosal portion ing in the cat some 60 and 40 per cent respectively It seems reasonable to assume that the maximal blood flow capacity of the intestinal smooth muscle portion does not surpass that of the skeletal muscles considering their respective metabolic levels in activity If this assumption is correct it follows that the glandular mucosal portion must have a maximal blood flow capacity of the order of some 500 ml/min/100 g of tissue This figure is in fact not a priori surprisingly big since the blood stream of the mucosa in contrast to the situation in muscles serves not only as a nutritional supply but also as a source of raw material for glandular cells with a high secretory capacity It is also a transport system for absorbed material Furthermore the range of blood supply coincides fairly well with that of other glandular tissues like the salivary glands To what extent this flow passes possibly existing shunt vessels is not known at present

The above mentioned changes in blood flow which reflect the shifts in tone of the resistance vessels were regularly correlated with changes in regional blood volume and CFC in their turn reflecting shifts in tone of the capacitance vessels and precapillary sphincters respectively In agreement with JOHNSON s findings on the dog s intestine (1959 1960) CFC of the cat s intestine was found to be definitely greater than that of the skeletal muscle (PAPPENHEIMER 1953 COBBOLD et al 1963) It is true that the relatively few CFC figures given by Johnson are definitely lower than those in the present study but so are his blood flow values perhaps partly a species difference However when

correlated with the curve in Fig 4 which relates blood flow to CFC Johnson s blood flow CFC figures coincide well with the lower left end of the curve

The greater CFC/blood flow ratio of the intestine as compared with the skeletal muscle which — as mentioned earlier — in all probability is even greater than that shown in Fig 4 calls for some remarks The mucosal portion is specialized for secretion and absorption functions that for a given blood supply may be predicted to require an especially big capillary exchange surface or fractional pore area It is known from many morphological studies that the gastrointestinal mucosa has an especially dense capillary network. Again it should be recalled that great differences in vascular architecture may exist between the smooth muscle and the mucosal portions of the intestine the latter portion being probably far better vascularized There are good reasons for believing that this is true for the capillary section too implying that the capillary surface area per unit tissue weight should be far bigger for the muco al portion If it is assumed that CFC of the intestinal smooth muscle portion like its maximal blood flow capacity does not surpass that of skeletal muscle it would follow that CFC of the mucosa in reality is about twice as great as indicated in the diagram of Fig 4 being then around 1 at maximal vasodilatation Such a filtration capacity is in fact of a similar order of magnitude as that of the renal glomeruli also about 1 if glomerular filtration like CFC is expressed in ml/min/mm Hg/100 g of renal tissue

On the basis of the data shown in Fig 4 and the considerations outlined above it is thus tempting to assume that the higher CFC/blood flow ratio of the intestine reflects a capillary network that in both relative and absolute terms is denser than that of skeletal muscle This would mean that a given blood flow volume at least within the intestinal mucosa is spread out over a definitely bigger capillary surface area passes this surface area correspondingly slower and that the average diffusion distance (blood tissue cells) is shorter all factors that will facilitate exchange It should however be realized that the higher CFC/blood flow ratio may be due in part to a greater number of pores per unit capillary area or/and to somewhat bigger dimensions of these pores There is in fact good evidence that the latter factor (SWANN *et al* 1961) may at least partly explain the very high CFC values of the intestine Whatever the case the capacity of capillary transfer both by means of filtration and diffusion must indeed be very big From a pathophysiological point of view this would mean among other things that even slight increases in mean capillary pressure in the mucosa can rapidly result in marked edema With regard to skeletal muscle it has been suggested that the autoregulation of the vascular tone which is essentially confined to the precapillary resistance vessels helps to protect the capillary level from accidental pressure increases (FOLKOW and OBERG 1961) As mentioned the intestinal vessels show considerable autoregulation and it was repeatedly observed in the present experiments that these active chan es of tone must take place mainly on the precap llary side

as mean capillary pressure to judge from an unchanged isovolumetric equilibrium was only little affected when arterial inflow pressure was changed In such a way these purely local adaptions of precapillary tone may be expected to protect against too drastic filtration losses and edema formation in the event of accidental increases in arterial pressure However many other factors may affect both the pre to postcapillary resistance ratio and the arterial and venous pressures which all contribute to determine mean capillary pressure Recently performed experiments (LEWIS *et al* 1962 FOLKOW *et al* 1962) indicate for instance, that under certain circumstances the vasoconstrictor fibre influence on the pre and postcapillary vascular sections in the intestine may lead to a situation in which an outward filtration over the big capillary surface ensues possibly accounting for the gross intestinal edema formation seen in some shock situations

With regard to the intestinal blood volume and its changes with vascular tone the present figures for resting blood content corresponded to some 7—9 ml /100 g of tissue (intestine and its mesentery) The results suggest that this blood content can be decreased some 30—40 per cent at maximal sympathetic activity and increased to about the same extent at maximal intestinal vaso dilatation Gross changes in transmural pressure within the venous capacitance section can of course considerably affect these figures which were obtained under experimental conditions in which both the arterial inflow and venous outflow pressure levels of the intestinal circuit were kept fairly constant The figure for blood volume expulsion at maximal vasoconstriction is in good agreement with that given by MELLANDER (1960) for the maximal vasoconstrictor fibre effect on the capacitance vessels of the muscle skin region of the cat's hind quarters

Th s study has been s pport d by grants f m the M d cal F culty Un er ty of G t borg (O L. and I W) f om th S hool f A ro p Med c ne th ough the European Off e Aerospace Res rch Un t d States A Fo (AF EOAR 61—47) and by grant H 5675 f m the National Inst tutes of Health Bethesda USA

AB Hässle Goteborg Sweden h s g ously o red the mployment of a technic n

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Stockholm 60 Sweden

Quantitative Aspects of Respiratory Reflexes from the Lungs and Chest Walls of Cats

By

CURT VON EULER and HARRY W FRITTS Jr[1]

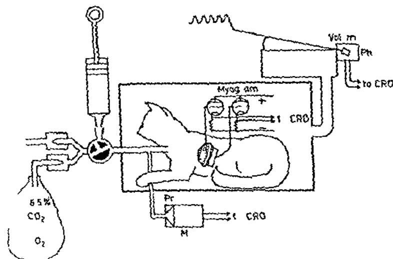
Recei d 27 July 1962

Abstract

EULER C V and H W FRITTS *Q a titative aspects of re pi atory refl es from the lung and chest walls of cats* Acta phys ol scand 1963 57 284—300 — Reflex regulation of the force of contraction of the respiratory muscles has been investigated and the relative importance of the cervical and thoracic dorsal root afferents has been compared with that of the gal nerves in cats anaesthetized with Dial After closing th t achea and inflating or deflating the l ngs to known volumes the int atracheal pressures developed during the respiratory efforts were measured before and after severing the vagi or the dorsal roots

The inspiratory pressure volume relat ons were approximately l near Th difference in lope obta ned b fore and after sectioring the agal nerves or dorsal roots p ovides an index of the gain of th control me hanisms The inh b tory eff ct exerted by the vagi v as almost lin arly related to th thorac c volume The dorsal r ots ex ted a fa litatory effect h ch d crease l with increas n thoracic volumes in a linear fash on Thus the afferents of both systems i creased the teepnes of the slope of the pressure olume curve the gain of the agal and of th dorsal root circuit be ng approximat ly the sam L ing el ctromyography ton c proprioceptive r flexes in the interc stal musculature were demon trated Release from in h bition of intercostal stretch reflexes was found follow ng high p nalizati n

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F gu 1 Arrang ment of xp rimental apparatus *M* tra n-gauge manometer *Ph* photocell. *CRO* cathode- ay osc ll sc pe

Of the many papers published on the sensory control of respiration the majority have dealt with the role of the pulmonary receptors in determining the depth and duration of the individual breaths Less attention has been given to the regulation of the force of contraction of the respiratory muscles That these forces are influenced both by the volume of the lungs and by stretch and tension of the respiratory muscles has been suggested in several papers Thus evidence has been presented indicating that man and animals select breathing frequencies which minimize either the force of contraction (MEAD 1960) or the work the muscles perform (ROHRER 1925 OTIS FENN and RAHN 1950 McILROY MARSHALL and CHRISTIE 1954 DAVIS FOWLER and LAMBERT 1956 ZECHMAN SALZANO and HALL 1958 SALZANO and HALL 1959) Imposing elastic loads has been f und to augment respiratory muscle force (CAMPBELL DINNICK and HOWELL 1961 CAMPBELL *et al* 1961) Pertinent also are the observations of DRAPER LADEFOGED and WHITTERIDGE (1959 1960) who found that the tracheal pressure is held remarkably constant during speach of steady loudness suggesting a fine regulation of the force of contraction of the muscles governing this pressure Proprioceptive reflexes in the chest wall have recently been described (GARCIA RAMOS and LÓPEZ MENDOZA 1959) and in a few earlier papers it has been demonstrated that sectioning the cervical and thoracic dorsal roots weakens respiratory movements (COOMBS 1918 COOMBS and PIKE 1918 1930 SEARS 1958 NATHAN and SEARS 1960) The intercostal muscle spindles and the m toneurones innervating the intrafusal muscle fibres of these proprioceptors have recently been found to be strongly engaged in the

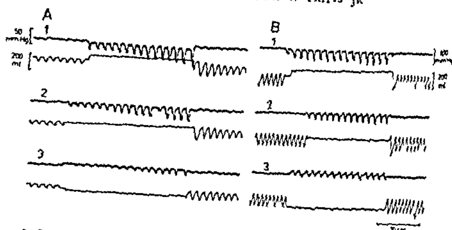


Figure 2 Records of p essure (upper) and volume (lowe) obtain d n a dece ebrate cat wh en the trachea was occluded at diff rent lung volumes Do vnward deflect on of the pres re curv dur ng occlus on denotes nsp ratory effort In 1 the cat breathed room a r and tl t chea was occluded at 17 ERV (1) 100 ERV (2) and 175 ERV (3) In B the cat b athed f 5 CO_2 n O and th t achea wa occl d d at 20 ERV (1) 120 ERV (2) and 230 ERV (3) Note that d ing t a t al occlu on tl e p essur reach d a plateau m rap dly when the cat breath d CO_2 than when h b eath d a

control of the intercostal muscles (CRITCHLOW and EULER 1962) yet little is known about the relative importance of dorsal root afferents for the control of respiratory movements as compared with the vagal afferents

The present experiments were designed to investigate the following related questions 1) To what extent do vagal afferents influence the force of contraction of the respiratory muscles? 2) To what extent is this force dependent on dorsal root afferents of the cervical and thoracic segments? and 3) Do the latter afferents participate in spinal reflexes or supraspinal reflexes? The first two questions have been approached quantitatively by measuring the intratracheal pressures developed by the respiratory efforts against the closed trachea after inflating and deflating the lungs to known volumes comparing the values obtained before and after sectioning the vagal nerves or the dorsal roots The regulatory mechanisms governed by these two afferent systems will be characterized 1) by their gain by which is meant the change in pressure (output) per unit change in thoracic volume (input) and 2) by the absolute values of the intratracheal pressures exerted at midposition The influence of the vagal and the dorsal root afferents will thus be expressed in the same units which permits a close comparison The third question has been studied by supplementing these procedures with electromyography Evidence for the presence of tonic proprioceptive reflexes in respiratory muscles of the thoracic wall will be presented confirming GARCIA RAMOS and LÓPEZ MENDOZA (1953)

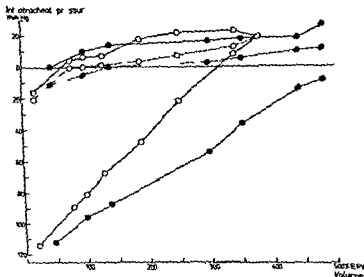


Figure 3 Pressure-volume diagrams of a decerebrate cat recorded before (open circles) and after (closed circles) bilateral vagotomy. Whole lines indicate peak pressures during respiratory efforts; hatched lines represent static pressures.

Methods

At the outset the cat received sufficient ether to allow cannulation of the trachea and isolation of the vagi. He was then either anesthetized with an intraperitoneal injection of Dial® (0.45 mg/kg) or decerebrated at a precollicular level. Those cats used for studying the effect of dorsal root section underwent laminectomies from C-3 to L-1.

With the operative procedures completed the cat was transferred to a table containing the apparatus sketched in Fig. 1. As shown in the drawing the cat lay in a body plethysmograph connected to a Krogh spirometer. A vane attached to the bell of the spirometer moved in a beam of light impinging on a photoelectric cell, thus rendering the output of the cell proportional to the cat's inspired and expired volumes. By deflecting one beam of a four beam cathode ray oscilloscope this output provided a record of ventilation. A second beam of the oscilloscope followed the intratracheal pressure measured by a strain gauge manometer. A third beam recorded the electrical potential (EMG) developed between a needle thrust into the fibers of an inspiratory intercostal muscle and a reference electrode clamped to the skin. The fourth beam followed the output of a capacitance which integrated the EMG.

Each experiment began by allowing the cat to lie quietly in the plethysmograph until the temperatures in the box and in the spirometer had attained steady levels. The resting ventilation was recorded then the cat began to breathe a mixture of 6.5% CO_2 in O_2. When a steady minute volume of ventilation had been achieved the expiratory reserve volume (ERV) was determined by the method of Van Liew (1954). This involved closing the tracheal cannula, applying suction until the intratracheal pressure fell to −40 mm Hg and measuring the difference between the final volume and that which had existed at the end of expiration as the cat breathed spontaneously. Because sectioning the sensory pathways usually changed the ERV and because the

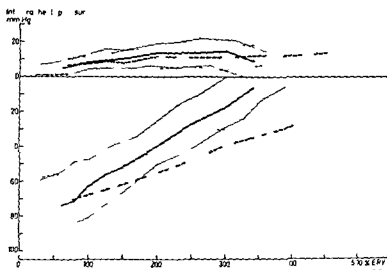


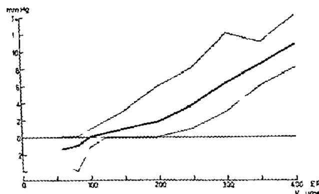
Figure 4 Pressure volume diagrams obtained in six decerebrate cats before (whole lines) and after (hatched lines) bilateral vagotomy Each thick line represents an average value the accompanying thin lines delineate the range

purpose of the study was to compare pressures at the same lung volume before and after resection it was necessary to inquire whether cutting the fibers also changed the residual volume (RV) We therefore estimated the functional residual capacity by the method of DUBOIS *et al* (1956) and after subtracting the ERV found that sectioning the afferent pathways did not alter RV Hence the presentation of the data was simplified by expressing each volume of deflation or inflation as a percentage of the ERV measured with the sensory pathways intact For example when the trachea of the cat was closed after applying —40 mm Hg suction the volume was 0 when closed at the end of a spontaneous expiration 100 % or when closed above this expiratory level greater than 100

Following the determination of the ERV the cat quickly returned to a steady level of ventilation whereupon at spaced intervals the trachea was closed at different levels of inflation and deflation During each period of closure the strain gauge measured the intratracheal pressure thus providing a record of the pressures produced by the inspiratory and expiratory efforts as well as the static level existing between the efforts when the muscles relaxed (Fig 2) In some cats the estimation of the static pressure was easy because a well defined interval separated the two phases of respiratory movements in a few there was no clear demarcation and the estimated value was of necessity only approximate

Results

Effect of Adding CO_2 to the Inspired Gas Mixture The decision to add 6 5 % CO_2 to the inspired gas mixture was based on three considerations First a preliminary study indicated that efforts developed against a closed trachea reached a steady level more rapidly when the cat breathed CO_2 This point is illustrated in Fig 2 A and B Second the steady level persisted for a period of



F gu e 5 D fferen e betw en th aver ge tat p es ur lum curves btain d before and aft ag tomy in 6 d er b te cats Th thuck lin represents the average d ff ence the thin l nes delineate th range P t v v lues den te redu ed tat c p essures aft v g tomy

fifteen or more seconds suggesting that at this degree of hypercapnea the effects of the changing levels of the blood gases during tracheal occlusion were mim mized And third the pattern of the results was not altered by the procedure whereas a cat tended to generate a larger pressure against a closed trachea when he breathed CO the direction of the change in the pressure volume relation produced by sectioning the afferent pathways was the same as when the cat breathed ambient air

T bl 1

Prep t on	Insp rat ry p essure t mid pos t on (100 ERV) mm Hg	Ga n of v gal circuit. (D ff en n l pe f pres sure olum dia grams before d after ag t my) mm Hg/100 ERV	G n of d rsal root ircuit. (D fferen e n sl pe f pres- ur olum dia grams bef e and fter hiz tomy) mm Hg/100 ERV
6 decer b te ats Dorsal oot ta t	64	10	—
6 Dial at D rsal roo nt ct	4	5	—
3 Dial t Dorsal roots ut	28	4	—
6 D al cats V g ut	49	—	5

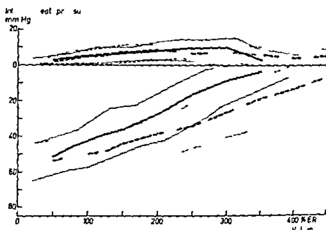


Figure 6 Pressure volume diagrams obtained in 6 anesthetized (Dial) cats before (whole lines) and after (hatched lines) bilateral vagotomy Each thick line represents the average value the accompanying thin lines delineate the range

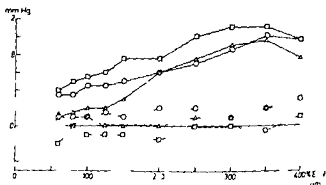
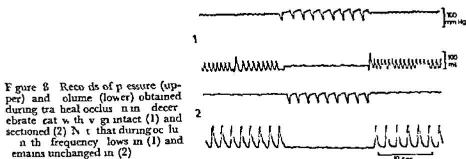


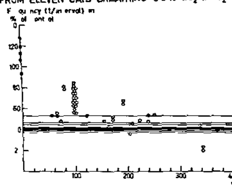
Figure 7 Differences in inspiratory pressures (whole lines) and in static pressures (hatched lines) before and after bilateral vagotomy in three anesthetized (Dial) cats with dorsal roots sectioned

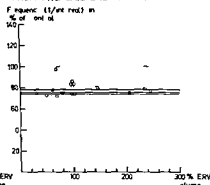
Effect of Vagotomy on Inspiratory and Expiratory Pressures The static pressure and the peak inspiratory and expiratory pressures developed against a closed trachea are depicted for a single decerebrate cat in Fig 3 In contrast to the approximate symmetry of the inspiratory and expiratory limbs of the pressure volume diagram produced by voluntary effort of conscious man (RAHN *et al* 1946) the cat generates much greater inspiratory than expiratory pressures This asymmetry observed in all of the cats studied was not influenced by the body weight by whether the cat was prone or supine or by the type of anesthesia

Fig 3 also shows the effect of vagotomy on the inspiratory and expiratory limbs of the pressure curves Severing these nerves allowed the cat to develop



F gure 8 Reco ds of p essure (upper) and olume (lower) obtained during tra heal occlus n in decer ebrate cat w th v gi intact (1) and sectioned (2) N t that during oc lu n th frequency lows in (1) and emains unchanged in (2)

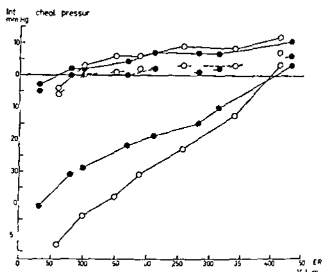




F gu 9 R lat on betw en th lum of th lung d th length of th espiratory cy l in 11 d reb t cats breathing CO (A) and in 5 dece brat cats breathing amb ent air (B) Th cycl length during tr heal occlusion is exp essed a a percentag of th length with th trach pen Th solid h ri ontal lines ep es nt th verag cy l lengths of th ind v dual cats after g t my W th th vag severed th cy l is indepe d nt f th lung lume.

greater inspiratory pressures suggesting that the vagal fibers had exerted an inhibitory effect The rise in pressure was particularly marked at large volumes of inflation and in a few cats the pressure volume curves obtained before and after vagotomy crossed at a volume close to midposition (100 / ERV) suggesting that the vagi inhibited contraction at levels above this value and facilitated contraction at levels below it Further vagotomy shifted the static curve in the direction of a diminished rigidity of the thorax and in most of the cats displaced the expiratory limb in the same direction as the static curves (Van Liew 1954)

The average curves of insp ratory and expiratory pressures obtained before and after vagotomy in six decerebrate cats are shown in Fig 4 Because the pressure peaks were read in relation to the corresponding static pressures (Fig 5) the curves represent the changes in pressure actually produced



F gu e 10 Pres ure olume d agrams of a vagotom ed anesthetı ed (D l) cat stud ed before (open c cles) and after (closed cı cles) serverıng the dorsal oots from C-3 to T 12 Whol lines ınd at peak pressu es dur ng respiratory effo ts hatched lines rep esent tat c pressures.

muscular efforts It can be seen that each average curve ıs approximately linear and has therefore an almost constant slope The difference ın slope before and after vagotomy provides an index of the gain of the vagal system The gain and the inspiratory pressures at midposition are given in Table I

Studies before and after vagotomy were also performed in cats anesthetized with Dial While both the gain and the inspiratory pressures were reduced (Fig 6 and Table I) the release from inhibition following vagotomy was similar as that observed in decerebrate cats

The observation that vagotomy changed the gain of the circuit raised the possibility that this alteration was mediated by muscle spindles coupled to γ efferents carried in the intercostal nerves To test this possibility pressure volume curves were obtained in three cats in which the cervical and thoracic dorsal roots had been sectioned but in whom the vagi were intact Again vagotomy changed the gain of the circuit in a fashion similar to that seen in cats with intact dorsal root However after rhizotomy the pressure volume curve was shifted downward to a lower level (Fig 14) Thus it can be concluded that the gain of the vagal system seen after sectioning the dorsal roots (Fig) was not mediated by the loop of efferents and muscle spindle afferents

The present studies also provided an opportunity for observing the well known change in respiratory frequency which vagotomy induces Closing the trachea in intact cats alters the frequency while the same maneuver in vagotomized cats changes the frequency scarcely at all (TROELSTRA 1960) This phenomenon is illustrated for a single cat in Fig 8 Fig 9 depicts the changes

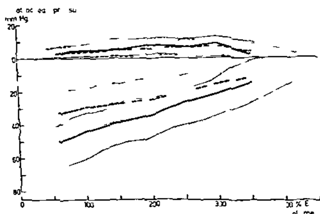
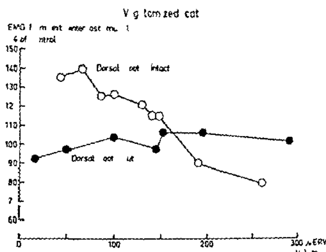


Figure 11 Pressure-volume diagrams of 5 vagotomized anesthetized (Dial) cats studied before (whole lines) and after (hatched lines) sectioning the dorsal roots from C-3 to T 12 Each thick line represents an average, the accompanying thin lines delineate the range

in frequency induced by occluding the trachea in two groups of cats one comprising eleven animals breathing CO_2 and the second 5 animals breathing air The graphs show that the change in frequency in both groups was a function of the lung volume In this regard it is interesting that the difference in frequency between intact and vagotomized animals is greatest at small volumes and least at large volumes (HESS 1936 TROELSTRA 1960) Thus within a considerable range the vagal influence on frequency is opposite to the control of the inspiratory force (TROELSTRA 1960) in that the frequency is increased when the force is reduced While this vagal effect on force is probably governed by slowly adapting pulmonary receptors which discharge in proportion to lung volumes (ADRIAN 1933 KNOWLTON and LARRABEE 1946 WIDDICOMBE 1954) the effect on duration of the respiratory cycle may depend more on receptors responding to deflation (ADRIAN 1933 KNOWLTON and LARRABEE 1946 PAINTAL 1955 1957)

Effect of Severing the Dorsal Root Fibers A preliminary study indicated that cats anesthetized with Dial withstood laminectomy better than those which were decerebrate and for this reason the effect of severing the dorsal roots was investigated in Dial cats alone Each cat had been vagotomized

Sectioning the dorsal roots reduced the pressures the cat generated This can be seen in Fig 10 which depicts the pressures developed before and after rhizotomy in a single cat The average results in 6 cats are shown in Fig 11 The difference between the inspiratory pressures exerted by the vagotomized and the vagotomized rhizotomized preparation indicates that increasing length of the inspiratory muscles as occurs when the thoracic volume decreases facilitates the inspiratory motoneurones by way of the dorsal roots Thus in contrast to the inhibiting effect of the vagal fibers the thoracic and cervical



Figur 12 Relat n of the lung volume to the integrated electr cal act v ty of an n p atory muscle The bservat ons w e made during tracheal occlus on in a v gotom zed cat anesthetized w th D al Open c cles p esent data r corded befo and clos d cycles afte sect on g th dorsal roots f om C-3 to T 12

dorsal roots appear to mediate a facilitation The dorsal root circuit is such that the slope falls from 13 to 8 mm Hg/100 % ERV when the roots are sectioned These gives a gain of 5 mm Hg/100 % ERV which is equal to the gain of the the vagal system (Table I)

In these cats with all of the sensory fibers sectioned the pressure developed by the animal still varied with volume This may be due to either of two factors 1) the fact that the force of contraction of any muscle increases with increasing length of the muscle fiber 2) the fact that the fraction of the muscle force applied to changing the volume of the thorax may depend on the degree of expansion of the thoracic wall

Electromyographic Studies of Segmental Reflexes The electromyogram provided information which supplemented the measurements of pressure and volume Whereas the relation between pressure and volume depended on (a) the motoneurone activity (b) the length of the muscles and (c) the attachment of the muscles to the chest wall the electromyogram provided an index of motoneurone activity alone Thus the technique afforded an opportunity to investigate whether the variations in pressure developing before and after rhizotomy were caused by changes in neural activity or just by the mechanics of muscle contraction The electromyograms were recorded from the inspiratory external intercostal muscles Fig 12 depicts the results of a typical experiment in a vagotomized decerebrate cat Each plotted point represents the average integrated electrical activity of the muscle per inspiration expressed as percentage of the average activity evident during spontaneous unrestricted breathing The averaged activity of 3—4 clamped efforts were thus compared with

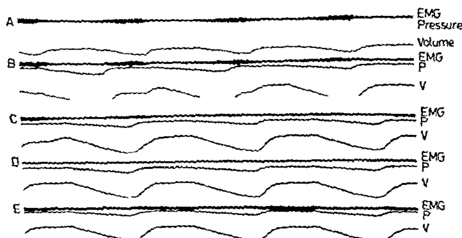


Figure 13 Effect of blocking the cervical spinal cord of a vagotomized decerebrate cat A spontaneous breathing *B—D* forced artificial hyperventilation. *E* spinal cord blocked at C ventilation same as in *B—D* See text.

the averaged activity of 4—6 unobstruced inspirations before and after each clamping The curves demonstrate that the electrical activity decreased with increasing thoracic volume After sectioning the dorsal roots in the cervical and thoracic region the expansion of the chest wall no longer influenced the electrical activity Nor did clamping the trachea influence the electromyogram as it did when the dorsal roots were intact Thus the dorsal roots reflexively enhanced the motoneurone activity at small and moderate thoracic volumes and inhibited the activity at large volumes The activity equaled that of unobstructed inspiration (100 %) at a volume of about 150 % ERV the point reached at the peak of the unrestricted tidal volume

Similar observations have been made when recording from single motor units although most of those tested were found to have a rather narrow range within which their frequency of firing followed changes in thoracic volume The dependence of motoneurone discharge on degree of inflation could be abolished by cutting the dorsal roots Thus the excitability of the spinal motoneurones in vagotomized cats is reflexively influenced by the position of the chest wall an effect which apparently is mediated by proprioceptive fibers carried in the intercostal nerves Manifest stretch reflexes were not seen under these experimental conditions After high cervical spinalization however reflex contractions could be elicited by expansion of the chest wall A typical experiment showing this is depicted in Fig 13 The segment of the record labelled A shows the vagotomized decerebrate cat breathing spontaneously The EMG activity was synchronous with inspiration the latter inscribing a downward deflection on the volume curve In B the cat was subjected to positive pressure hyperventilation by means of a Starling pump With continued hyperventilation

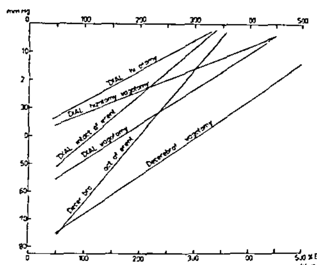


Figure 14. Summary of the effects of anesthesia, vagotomy and rhizotomy on the pressure-volume diagram of the inspiratory muscles

(C and D) the muscle ceased firing presumably because of the reduced hypercapneic drive. Indeed if at this point the pump was stopped briefly the cat was apneic. The cord was then blocked at the level of C-1 by injecting 3 ml of 2 % Xylocaine (Astra). Immediately afterward artificial ventilation was reinstituted (13 E) and now the unit — instead of firing with inspiration as it had previously — was active during expiration when the inspiratory muscle fibers were stretched. Thus a stretch reflex was elicited. This reflex was also occasionally evident in cats with unblocked cords when the blood pressure had fallen or when the cat was under deep anesthesia. Otherwise inflations and deflations did not provoke manifest stretch reflexes from the chest wall.

Discussion

The reflex and closed circuit control of muscle contraction can be assessed by plotting length tension curves before and after sectioning the sensory pathways and then using these curves to calculate myotatic loop gains (Granit 1958, Matthews 1958, Pompeiano 1960). This approach necessitates attaching an isolated muscle or portion of a muscle to a mechano-myograph, a procedure which if applied to a respiratory muscle would allow measurement of the force of contraction but not the effect of this force on the motion of the chest wall. Further isolation of each respiratory muscle may not be possible and the muscle portions that are available for myography, such as the diaphragmatic slip of Head (1889), are probably not representative of the entire respiratory musculature because the distribution of proprioceptors, while not precisel

known is thought to be non uniform such receptors probably being scarce in the diaphragm (DOGIEL 1902 GREGOR 1904 MASUMOTO 1934 a b YASARGIL 1962) and plentiful in the intercostal muscles (KERSCHNER 1888 COOPER 1960 BARKER 1962 CRITCHLOW and EULER 1962) By substituting volume and pressure measurements for determinations of length and tension the afferent control of the respiratory force may be analyzed according to the above mentioned principle in spite of the mentioned difficulties Measuring the pressures developed against a clamped trachea not only allows the muscles to function in their natural positions but also provides a means of studying the net effect of the inspiratory and expiratory muscles These advantages are somewhat offset however by the non linear relations between intratracheal pressure and muscle force and between changes in thoracic volume and muscle length Thus the shapes of the volume pressure diagrams which are summarized in Fig 14 reflect not only the force of contraction of the respiratory muscles but also a factor introduced by the method of measurement

Application of this approach revealed that vagotomy increased the pressures developed by the inspiratory muscles Such an increase might be mediated in any one of three ways First the respiratory drive might be greater with the vagi cut While this mechanism cannot be discounted its importance is questionable since following tracheal occlusion the developed pressures rapidly reached a plateau Thus if the respiratory drive were less than maximal the magnitude of the pressure should have continued to slowly increase The second possibility pertains to the vagal effect on the duration of the inspiratory effort Indeed in one cat this seemed to be the explanation because while the initial rate of change of electrical activity during inspiration as well as of the induced pressures was the same before and after vagotomy the latter inspiratory effort continued for a longer time (cf LARRABEE and KNOWLTON 1946) While this factor undoubtedly played a role in the other cats studied it did not completely explain the difference observed Therefore we are left with an increased frequency of discharge of the alpha motoneurones as the best possibility Since this increase could be elicited with the dorsal roots sectioned it seemingly did not depend chiefly on the external loop of gamma motoneurones and muscle spindle afferents

Sectioning the dorsal roots of the vagotomized cats lowered the pressure changes produced by muscle contraction Electromyography provided evidence that alpha motoneurone output is facilitated by impulses transmitted through the dorsal root fibers thus confirming the conclusion of GARCIA RAMOS and LOPEZ MENDOZA (1959)

Finally the data summarized in Fig 14 allow the first two questions posed in the introduction to be answered in the following way (1) The vagi exert an inhibitory influence on muscle contraction and (2) the dorsal roots facilitate contraction Both effects increased the steepness of the volume pressure diagram although the curves remained almost linear Besides the changes i it is

also apparent from Fig 14 that there are important parallel shifts of the diagrams along the ordinate This suggests that in addition to the volume dependent changes in pressure and gain the afferent systems maintain a steady level of excitation which does not change with the thoracic volume Both the gain of the afferent systems and the level of maintained excitation unquestionably depend on the respiratory drive These two factors may therefore be of importance for controlling the rate of rise of inspiratory force and hence for the duration and depth of an adequate breath

The third question raised in the introduction can be answered in the affirmative because proprioceptive reflexes could be elicited at segmental levels of the cord Of particular interest is the relea e from inhibition of the intercostal stretch reflexes following severance or blockage of the spinal cord in the upper cervical region This suggests that the responses to inflation and deflation before spinalization are also true segmental stretch reflexes although a participation of reflex arcs involving medullary structures should not be neglected Similarly release of splanchnic to intercostal and intercostal to-intercostal reflexes from tonic inhibition maintained by medullary structures have been demonstrated following high spinalization (DOWNMAN 1955 DOWNMAN and HUSSAIN 1958) Possibly the inhibition of the stretch reflexes in the chest wall is maintained from the same supraspinal structures Whether this descending inhibitory activity changes in phase with respiration and thus appears mainly in the expiratory phase or whether the inhibition is governed according to ›t principles remains an open question

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Acta physiol scand 1963 57 301—308

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Exchange of Phosphate-Groups between Inorganic Phosphate and Adenosine Triphosphate in Red Blood Cells

By

CARL HENRIC DE VERDIER

R ce ved 24 July 1962

Abstract

DE VERDIER C H *Excha ge of phosphate g ups between inorganic phosphate and adenos e triphosphat in r d blood cells* Acta physiol scand 1963 *57* 301—308 — In hemolysates of human red blood ells the e is a ap d ex hange of phosphate groups between norganic phosph te and the t rm nal phosphat group of adenosine triphos phate The exchang is dependent upon the phosphoglyceraldehyde dehydrogenase and the phosphoglyceryl kinase reactions s nce pho phoglyc aldehyde 3 phosphoglycerate and DPN are essential for the exchange react on The implication of thi finding is that studies of the ncorpo ation of inorganic labelled phosphate into d ffer nt compounds of the rythrocyte cannot n general be used to draw conclus ons about the glucose metabolism of ed cells

Glucose breakdown in erythrocytes during normal and pathologic conditions has been studied by PRANKERD (1957) by investigating the incorporation of P labelled phosphate into various compounds It has been supposed that intracorpuscular inorganic phosphate enters the metabolism in the phosphoglyceraldehyde dehydrogenase reaction GOURLEY (1952) found that ATP was the first compound to become labelled Later PRANKERD and ALTMAN (1954) considered that 2 3 diphosphoglycerate was labelled prior to ATP but GERLACH *et al* (1958) TATIBANA *et al* (1960) BARTLETT (1961) and ZIPARSKY *et al* (1962) have by incubations with iP [1] for shorter periods definitely proved that ATP has a higher specific activity

Th following bbr iati ns are used iP in ganic o tho-phosph t ATP denos n t phosph t ADP d os ne diphosph t DPN nd DPNH oxidized and red d form espect ly of d pho ph pyr di nucl ot d PGAl nd PGAlD phosphoglyceraldehyde and t d hydrogenase PGK phosphoglyceryl kinase

The purpose of this investigation is to show that labelling of ATP may to a high extent be the result of exchange reactions and does not in general permit conclusions about glycolysis in the red cells

Methods

High molecular weight fraction of erythrocytes Hemolysates were obtained by repeated freezing and thawing of washed erythrocytes 2 ml of hemolysate was applied to a 20 × 2 8 cm Sephadex G 25[1] column and the high molecular weight compounds (hemoglobin enzymes etc.) were obtained by elution with 0 15 M Tris-chloride pH 7 2 The hemoglobin content was adjusted by dilution with Tris-chloride to 1 5 g

Incubation After preheating to 37 C the high molecular weight fraction of erythrocytes was incubated at 37 C under agitation with different cofactors and intermediates as stated in the individual experiments Withdrawn samples were inactivated by heating for 30 sec in a boiling water bath

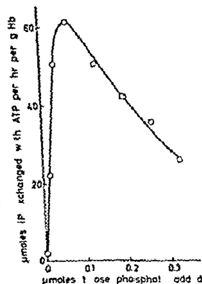
Exchange rate The exchange rate was measured by determining the chang in distribution of radioactive phosphorus between inorganic and organic form For this purpose a slight modification of the method of LINDBERG and ERNSTER (19 6) was used The exchange rates were calculated on the assumption that no net synthesis of compounds occurred This is considered to be likely as in the iP ATP system no ADP was present and as in the ATP iI system the exchange occurred with both DPN and DPNH As the exchange is fairly low (< 70 /) under the experimental conditions used no corrections were made in the calculations for reformation of labelled precursor from the labelled product

Chemicals All chemicals were of reagent grade 3-phosphoglycerate Ba salt was purchased from California Corp for Biochem cal Resea ch, Los Angeles and converted to the Na salt. Triose phosphates (a solution containing 2 75 mg/ml of each dihydroxy aceton phosphoric acid and glycerinaldehydephosphoric acid) was obtained from Boehringer & Soehne GmbH Mannhe m West Germany P inorganic phosphate (PBS 1) was obtained from The Radiochemical Centre Amersham England ATP was prepared from iP by using the exchange reaction described in more detail in the section on results It was purif ed by elution through a Dowex 1 column (DE VERDIER and KILLANDER 196?) and desalted according to SMITH (1960, The concentration of ATP in the preparation was determined spectrophotometrically and before use it was diluted with unlabelled ATP

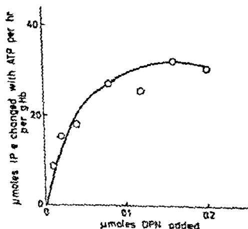
Results

Exchange between iP and ATP If the exchange between iP and ATP is the result of exchange in the phosphoglyceraldehyde dehydrogenase (PGAlD) and the phosphoglyceryl kinase (PGK) reaction it should be dependent on cofactors and substates for these reactions That this is the case is illustrated in Fig 1—3 Diphosphopyridine nucleotide and PGAl are thus both necessary for the exchange reaction The triose phosphate mixture used instead of PGAl seems to have an inhibitory effect in high concentration Fig 3 illustrates an experiment in which rising concentrations of pyruvate promotes the exchange probably by increasing the DPN/DPNH ratio through the lactic dehydrogenase reaction

[1] The gift of Sephadex G from AB Pharmacia Uppsala, is grat full acknowledged.

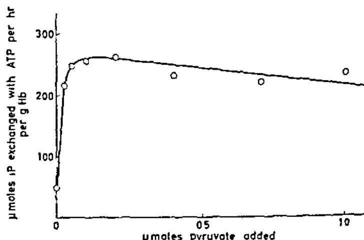


F g 1 Relat n betw en concent at on of triose phosphate nd P³² ATP e change Incubat on mixtu DPN 0 1 µmole MgCl 0 5 µmol iP³² 0 5 µm l ATP 0 5 µm l duced glutath 0 5 µmole h gh m l cular ght fr ct on of erythrocytes 150 µl final ol m 210µl

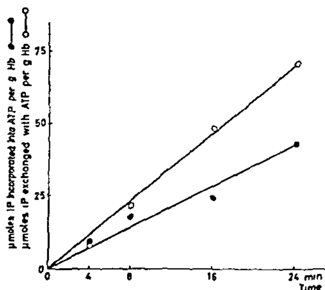


F g 2 R l t n betw n concent t on f DPN and P³² ATP ex hang Incuba i n mixtu mila to that described in F g 1 ex ept th t th am nt of t ose ph sphate was nstant (0 065 µm l) and th m nt f DPN a ed

Comparison f r action rate by exchan with ATP and by direct formation of ATP Two incubation media were mixed one containing ATP the other instead an equimolar concentration of ADP The transfer of iP ³² to organic form was compared for the two media (Fig 4) It is evident from the figure that with the substrate concentrations used iP is more rapidly converted to organic form by the exchange reaction That in both media the labelled organic compound is ATP has been shown chromatographically (DE VERDIER and KILLANDER 1962) At the end point only 18 of P³² was transferred to ATP which explains the almost linear relationship between time and incorporation

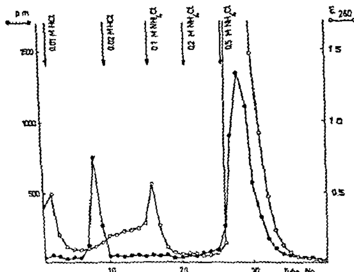


F g 3 Relat on between concentration of pyruvate and iP³ ATP exchange Incubat on mixt re similar to that described in F g 1 and 2 Constant amount of triose pho phate (0 065 μmol) and DPN (0 1 μmol)

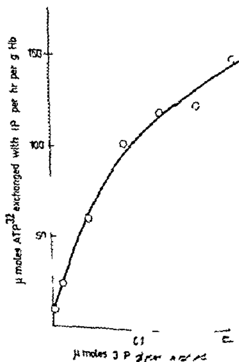


F g 4 Comparison of the labelling of ATP btained by di ect synthes (sol d c cles) a d exchange (open les) Fo the P ATP exchange the f llowing in cubat on m ture was used DPN 0 1 μmole MgCl 0 5 μmole P³ 0 5 μmole ATP 0 5 μm l red ced gluta th on 0 5 μm le triose phosphat 0 065 μm l Na pyru at 0 1 μm l h gh molecular w ght fr ct on of eryth ocytes (0 7 g Hb) 150 μl Final v l m 200 μl For d rect synth s ATP wa replaced by eq molar amo nt of ADP

The position of P ³ in the ATP molecule To elucidate this 0 9 μmoles ATP was mixed with 5 0 μmoles unlabelled ATP and hydrolyzed for 10 min in 0 01 M HCl at 100 C in a sealed glass tube After evaporation of the hydrochloric acid the residue was chromatographed according to DE VERDIER and KILLANDER (1962) The elution curves show that although both AMP and ADP were formed by the hydrolysis no measurable amount of radioactivity was found at the ADP peak (Fig 5) This indicates that it is mainly the terminal phosphate group in ATP that has been labelled



F g 5 ATP* labell d through th exchang eact on was partually hydr ly ed and th p oduct were separat d on a Dowex 1 olumn P ADP and ATP wer luted by 0 001 M HCl 0 0 M HCl — 0 1 M NH Cl and 0 5 M NH Cl respect ly



F g 6 Corr l t n betw n c n ntrat n f 3 P gl cer and P ATP* hang Incuba mixtur DPN 0 1 μm l MgCl 0 5 μmol P 0 5 μm l ATP* 0 5 μm l ed ed glutath n 0 5 μm l h gh m l ula w ight f a ti n f ry h ocytes 150 μl final l m 210 μl

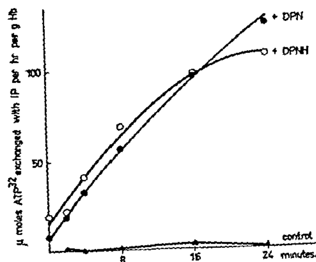


Fig 7 Effect of DPN and DPNH on iP ATP* exchange In control DPN and DPNH were omitted. The other two curves represent experiments in which 0 1 μmole DPN or DPNH was added 0 1 μmole 3-P-glycerate is added in all three series Other ingredients same as Fig 6

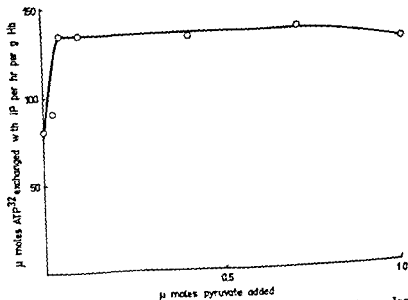


Fig 8 Correlation between concentration of pyruvate and iP ATP* exchange Incubation mixture similar to that described in Fig 3 except that labelled ATP and unlabelled P were used Triose phosphate was replaced by 0 1 μmol 3 P glycerat

Exchange between ATP and iP* A series of experiments has also been performed with this reversed type of exchange The results are illustrated by Fig 6—8 DPN or DPNH and 3-phosphoglycerate are essential Pyruvate promotes the reaction although to a lesser extent

Discussion

The PGAlD reaction and the nucleoside phosphorylase reaction have both been proposed to be the substantial biochemical background for the transport mechanism of iP through the erythrocyte membrane (ZIPURSKY and ISRAELS 1961) If the PGAlD reaction is responsible it would explain the rapid label ling of ATP Recent investigations however indicate that there is a special carrier system for iP through the membrane (SCHAUER and HILLMAN 1961 ÅGREN 1961)

According to evidences put forward by *e g* KOEPPE BOYER and STULBERG (1956) an enzyme substrate DPN (not DPNH) complex is reacting with iP and this may be the explanation for the stimulating effect of addition of pyruvate upon the exchange The more pronounced effect obtained when the reaction is investigated with the iP system than with the ATP^{32} system cannot be ex plained at present

Phosphate exchange has been described in a pure PGAlD system by OESPER (1954) and according to RACKER (1961) exhcange is also observed in a PGAlD PGK system No details are given however

Experiments described in this communication give details for preparation of more or less highly labelled ATP without the need for expensive com mercial enzymes (PLEIDERER 1961)

The main purpose of this paper is however to emphasize the difficulties involved in using incorporation of P into different compounds as an indicator for errors of glycolysis in the red cell ATP is the most important carrier in transphosphorylating reactions in the red blood corpuscle Since in experiments with P the labelling of ATP is not directly dependent upon the net flow of metabolites via the PGAlD and the PGK reactions and since the labelling to a high extent can be influenced by the redox equilibrium in the cell it seems to be difficult to interpret the results of P incorporation experiments The experiments reported here are made with hemolysates *in vitro* and it is not easy to evaluate in the living erythrocyte the proportion between ATP formed by exchange reaction and ATP^{32} formed by *de novo* synthesis Although the pyruvate /lactate ratio is low in the cells the exchange is certainly of importance

Th kilful t chni al ass tan f Mrs BRITT MARI ALGOTS gr t fully a kn wledged This estig ti n has be n po s ed by the Sw dish Med cal Res h Counc l

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Excitatory and Inhibitory Effects of Vagus Stimulation on Gastric Motility in the Cat

By

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Abstract

Martinson, J. and A. Muren. *Excitatory and inhibitory effects of vagus stimulation on gastric motility in the cat.* Acta physiol. scand. 1963. 57. 309—316. — In acute experiments on cats gastric motor responses were recorded during vagal stimulation with a constant frequency but with different values of impulse duration and voltage. With very low values cardiac responses only were obtained. With higher values pure excitatory motor effects of the stomach were elicited, but when the values were increased above a certain point a gradual depression of the response was observed. After atropine the gastric motor responses were abolished and pure relaxation was the result of stimulations which previously had given rise to partially depressed excitatory responses. It is suggested that two groups of efferent fibres influencing gastric motility are present in the vagus nerve. According to the strength-duration diagram both fibre groups are of smaller caliber than the cardioinhibitory fibres, the inhibitory fibres being the thinnest ones. The excitatory fibres appear to be cholinergic whereas the inhibitory effect seems to be mediated by some other transmitter substance.

The effect of vagus stimulation on gastric motility has been subject to detailed studies since more than a hundred years. Most studies are concerned with the excitatory effect of vagus-stimulation, but there are reports on an inhibitory effect appearing under certain conditions during vagus-stimulation already from the later part of the 19th century. McSwiney (1931) has reviewed the different investigations on the subject up to that time. Various explanations

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have been proposed concerning the mechanism responsible for the inhibition According to one opinion represented by VEACH (1925) inhibition occurred when a high frequency or strength of the stimulation was used and this was related to the phenomenon called Wedensky inhibition This explanation was criticized by McSWINEY and WADGE (1928) who found that the type of response to vagus stimulation depended on the basal tone of the stomach contraction appearing when the tone was low and relaxation when the tone was high In 1936 HARRISON and McSWINEY concluded that *the inhibitory effect m ht* be due to adrenergic fibres in the vagal nerves since inhibition was not abolished by atropine This explanation seems to have been generally accepted and apart from a few reports usually in connection with related problems vagal in hibition has not been subject to much attention during the last decades ELIAS SON (1952 1954) was able to elicit gastric inhibition during stimulation of the orbital region of the brain stem in cats These effects which were found to be conveyed via the vagus diappeared after atropine GREEFF and HOLTZ (1956 a) working with an isolated vagus stomach preparation from guinea pig obtained inhibitory effects during vagus stimulation only in the presence of atropine after increasing the tone with histamine or barium chloride This in hibition was abolished by sympatholytic drugs

The present investigation was induced by the observation that gastric motor responses were difficult to obtain when the vagus was stimulated with the relatively high values of voltage and impulse duration which were chosen during studies on the effect of frequency variations The results have been briefly described in a preliminary report (MARTINSON and MUREN 1960)

Materials and methods

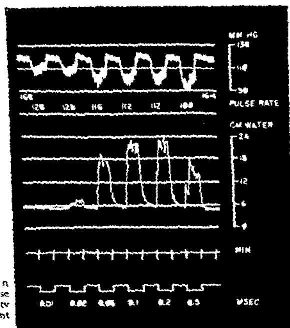
Experim nts were made on 30 cats anesthetized with chloralose and urethan (80 + 40 mg/kg body we ght) or with pentobarbitone (30 mg/kg) after one day s deprival of food

A tracheal cannula was inserted and the blood pressure manometer was connected to a carotid artery A latex ballon was introduced into the stomach via the oesophagus connected to a float recorder via a water manometer and inflated with 40 ml of air Both vagal nerves in the neck were carefully d ssected free from the sympathetic trunc and cut centrally The distal end of the right or the *left* vagus was *placed* on a b polar silver electrode and covered with cotton wool soaked in warm liquid paraffine

A stimulator giving square wave pulses was used (Grass model S4) The frequency was kept constant througho t the exp riment usually 4 impulses per s cond Voltage and impulse duration were varied stepwise according to a fixed scale (1 2 5 and 10 volts and 0 01 0 02 0 05 5 and 10 milliseconds respectively)

Results

Fig 1 gives the appearance of a series of stimulations with *different impulse* durations wiht the frequency and voltage kept constant at 4 imp/sec and 10 volts respectively With a threshold at 0 02 msec the responses successively in



F g 1 Hea t rate and gastric m t esponses to a se es of timulat n w th a tep ise increase of impulse dur t n Frequency nd intens ty f s mulation current kept onstant at 4/sec and 10 lt espect ly

crease in size to a maximum at 0 2 msec With further increase of impulse duration the motor responses are reduced in size Similar series were made with different values of voltage and the general appearance of each series was the same with a displacement towards higher values of impulse duration when lower voltage was used However when an intensity of less than one volt was used a reduction of the response was practically never seen even if the impulse duration was increased up to 10 msec or more From Fig 1 is further seen that the stimulation threshold for cardiac deceleration is lower than that for gastric motor excitation (See also Fig 4 which includes the strength-duration curve even for heart deceleration)

Fig 2 shows an experiment in which impulse duration has been kept constant (5 msec) and the voltage has been increased with small steps over a narrow range in order to illustrate how abruptly the increase and the decrease of the motor responses develop The last two stimulations in this series show that the excitatory response is not completely abolished even with a considerable increase of voltage

The results obtained were principally the same in all experiments Stimulation of the right and the left vagus gave similar results and the type of anaesthesia did not seem to be of any considerable importance As for the depression of the response with increasing voltage or duration different characteristics could be observed

a) reducti n of the max mal height of the response

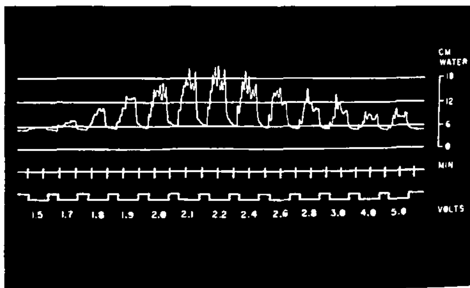
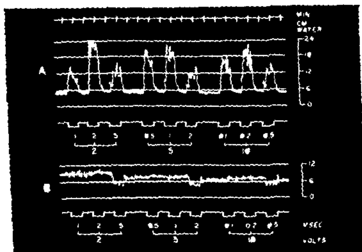


Fig 2 Gastric motor responses to a series of stimulations with a stepwise increase of voltage Frequency and impulse duration kept constant at 4/sec and 5 msec respectively

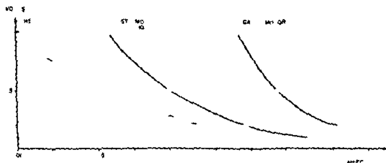
b) interruption of the initial rising phase

c) prolonged depression of the basal tone following stimulation These characteristics usually appeared in combination but they were not equally pronounced in the different experiments They have been used as criteria on the inhibitory effect (and are referred to as criterium a b and c) Most of these different types of changes are clearly seen in Fig 1 and 2

The observations seemed to indicate that an inhibitory influence appeared superimposed on the excitatory responses when voltage or impulse duration was increased above a certain value A further support to this explanation is the appearance of the record after administration of a dose of atropine sufficient to abolish the excitatory response (Fig 3) In this case there is a pure elevation of the basal tone During the 10 min that separate part A from part B atropine 0 1 mg/kg was given and after atropine the basal tone was increased by injection of a big dose carbachol (0 1 mg/kg) When the same series of stimulations as those given in part A were repeated a relaxation was obtained with stimulations which previously had succeeded the maximal responses The basal activity could also be increased by injection of histamine into the aorta at the level of the diaphragm The basal activity thus obtained was also depressed by stimulation with high intensity or duration These experiments were repeated with various modifications and it was always found that stimuli causing inhibition after atropine always corresponded to stimuli which prior to atropine had given rise to partially reduced responses In other words the threshold for inhibition was the same before and after abolition of the excitatory responses



F g 3 A Gast moto esponses to thre diff rent timulation series with variation of mpulse d r t n nd v ltag F equency constant at 4/sec B Sam experiment repeated after atrop n (tim interval betw en A and B 10 minutes)



F g 4 St ength-durat n curves t the th eshold al es f ex tators and of nh b tory effects f agus timulat on on gast m tility Thre bold lues fo decelerat on of the h art lso pres nted f r mp is o M an of 20 experiment

It was fi rther observed that the latency of the inhibitory responses was ne er more than 1—3 sec and often it was considerably shorter Since each experi ment included series of stimulations which usually covered a wide range of values for voltage as well as for impulse duration a strength-duration diagram could be dra n for each animal including one diagram for excitatory and one f r inhibitory thresholds

The geometric mean of the threshold values obtained from 20 cats in most of which stimulations were made on both right and left vagus is presented in Fig 4

Discussion

The method of recording gastric motility by means of a balloon has been subject to much dispute since the balloon is considered to act as a bolus and thus eliciting activities superimposed on the effect to be studied In present studies however the method has been considered satisfactory since the principal total gastric motor activity demand on the method is to visualize in terms of excitation or inhibition

The findings suggest that there are two types of efferent vagal fibres influencing gastric motility one excitatory and one inhibitory the latter with higher stimulation thresholds The relationship between the stimulation thresholds suggest that the caliber of the inhibitory fibres is considerably less than that of the excitatory ones which in its turn are of smaller caliber than the cardiac decelerator fibres As to the criterium b of inhibition (see above) the initial excitatory response followed by a depression supports this explanation *i e* the inhibitory fibres are of smaller diameter and thus of slower conduction velocity

The vagus nerves to the abdominal viscera is found to contain less than 3 000 efferent fibres or less than 10 % of the total count (AGOSTONI *et al* 1957) nearly all of which are unmyelinated and of small diameter No further investigation on the fiberspectrum seems to have been made however to which the present findings could be correlated

Inhibition as described here seems to imply a partial depression of motor activity no matter of what origin In the intact organism a similar inhibition might be obtained by decreasing the basal nervous tone by means of central nervous influence The inhibition described by ELIASSON seems to be of this type. That mechanism however could hardly be responsible for the inhibition described here since the vagus nerves were cut The efferent fibres stimulated could of course also be assumed to act on myenteric plexus nerve cells and elicit their inhibitory action there on the other hand the inhibition observed in the present investigations was not abolished by atropine

There seems to be no direct correlation between the results described here and those obtained by McSWINEY and WADGE (1928) who considered the effect of vagal stimulation to be mainly dependent on the initial tone However it must be kept in mind that when the basal tone is high there is less room left for further excitatory effects whereas inhibitory responses might appear quite easily The reverse is true in the case of low initial tone Furthermore the inhibitory responses obtained by McSWINEY and WADGE were usually preceded by a slight contraction which accords with criterium b above Their reports do not include an exact definition of the stimulation parameters used but it may be assumed that the inhibitory responses obtained by these workers corresponded to the high threshold stimulations of the present experiments The results described by VEACH seem to have much in common with those obtained here However he does not believe that there is a special group of fibres with

inhibitory effect on the stomach Instead he attaches most importance to the role of frequency variations and relates the inhibitory effects to the so called Wedensky inhibition which later on has been considered to be a form of exhaustion most likely of the transmission mechanism The inhibitions obtained by low frequency and high intensity stimulation however seem to be of the same character as those of the present investigations

The general opinion that excitatory fibres to the stomach are cholinergic (DALE and FELDBERG 1934 GREEFF and HOLTZ 1956 b) is supported by the present findings The inhibitory effect however is clearly demonstrable after a dose of atropine which abolishes the excitatory response a finding that indicates that this effect is of non cholinergic origin HARRISON and McSWINEY (1936) considered the inhibition to be elicited by adrenergic fibres although they underlined the fact that the inhibitory responses obtained by vagus stimulation were not of the same character as those obtained by stimulation of the splanchnic nerves

The short latency period indicates that no indirect humoral mechnism involving the adrenals is responsible for the inhibitory effects described here

The existence of sympathetic fibres in the vagus has been demonstrated previously by several authors (RANSON FOLEY and ALPERT 1933 and others) The results obtained by GREEFF and HOLTZ (1956 a) on isolated stomach preparations are also in accordance with this They obtained inhibitory effects on vagal nerve stimulation which were possible to abolish by sympatholytic drugs such as dihydroergotamine The vagal inhibition described in the present investigation was not abolished by this substance The influence of this and related substances however on adrenergic inhibitory effects is somewhat doubtful especially in the living animal and not so clear cut as the corresponding parasympatholytic effect of for example atropine The negative findings in this respect could threfore not be considered as conclusive and the problems requires further studies

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Acta physiol scand. 1963 57 317—327

From the Institute of Medical Biochemistry University of Oslo Norway

The Basal Content and the Induced Biosynthesis of Pyridine Nucleotides in the Rat Liver under the Influence of Insulin

By

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Abstract

ALERTSEN AA. R H N HAUGEN and E WALAAS *The basal content and the induced biosynthesis of pyridine nucleotides in the rat liver under the influence of insulin* Acta physiol scand 1963 *57* 317—327 — The basal content of pyridine nucleotides and the synthesis of the coenzymes induced by parenteral injection of nicotinamide have been investigated in the rat liver when exposed to the action of insulin adrenaline and ACTH The basal content has been investigated after injection of insulin or adrenaline and in alloxan diabetic rats The significance of the rather small variations in the basal content is discussed In the induced synthesis of DPN the injection of insulin or ACTH has been found to interfere with this process giving a decrease in the elevated content of DPN The hormone action has been related to the mechanism of increased turnover of the DPN molecule The administration of ethionine likewise decreased the elevated level of DPN The significance of a regulatory influence of hormones on the induced synthesis of DPN has been discussed

It has been proposed that the mode of action of insulin on energy metabolism is related to an effect on the content and turnover of pyridine nucleotides (CHAIN *et al* 1956) Only small variations have been recorded however in the total content or in the relative distribution between oxidized and reduced forms in the liver (GLOCK and MCLEAN 1955 HOLZER *et al* 1954) The evalu

Abbreviations used DPN diphosphopyridine nucleotide oxidized form
DPNH diphosphopyridine nucleotide reduced form
TPN triphosphopyridine nucleotide oxidized form
TPNH triphosphopyridine nucleotide reduced form

ation of these small variations depends upon the experimental procedure used With appropriate extraction procedures for the oxidized and reduced forms (CIOTTI and KAPLAN 1957) and with specific enzyme assay (CIOTTI and KAPLAN 1956 JACOBSON and ASTRACHAN 1957 HOLZER *et al* 1958) reliable results can now be obtained in these investigations

The basal content of pyridine nucleotides after the injection of insulin or adrenaline has been investigated in the present study Only small variations were obtained in insulin treated animals Adrenaline was found to be without effect Because of the conflicting results reported in the literature on the basal content of pyridine nucleotides in alloxan diabetes (GLOCK and MCLEAN 1955 HOLZER *et al* 1954 GREENBAUM and GREYMORE 1956) this problem was re investigated Essentially the same results were obtained as those reported by GLOCK and MCLEAN (1955)

It has been shown in the present work that insulin produces a decrease in the elevated DPN content in rat liver induced by the parenteral administration of nicotinamide This metabolic system which has been thoroughly investigated by KAPLAN *et al* (1956) is a valuable tool for investigating metabolic altera tions in liver cell metabolism The enzymes concerned in the biosynthesis have been investigated by PREISS and HANDLER (1958) Still many questions are unsettled however about the mechanism whereby nicotinamide induces synthesis of DPN *in vivo* *In vitro* addition of nicotinamide to liver tissue prepa ration does not lead to a synthesis of DPN

In the present study the effect of ACTH has also been investigated In ex periments of short duration a decrease in the elevated level of DPN was found this decrease being approximately of the same order of magnitude as with insulin

Material and Methods

Grown albino rats weighing 250—300 g were used throughout all the experiments

Production of alloxan diabetes Groups of rats were made alloxan diabetic by the injection intravenously in the tail of 0 15 ml of a solution containing 20 mg/ml (*i e* 3 mg alloxan) per 100 g rat in one single dose The rats which showed permanent glucosuria were selected for the investigations The animals were used in experiments 3—4 weeks after the injection of alloxan.

At the time of sacrifice blood sugar determinations were made In these animals a hyperglycemic level of blood sugar of the order of 200—400 mg/100 ml was recorded Blood sugar determinations were made by the glucose oxidase method (HUGGETT and NIXON 1957) Alloxan was a Merck product

Hormone injections Groups of animals were given insulin one hour before investigation These animals were given insulin Vitrum 0 1 unit per 100 g intravenously in the tail vein Other groups were given insulin Retard 0 1 unit per 100 g injected subcutaneously

Adrenaline was injected subcutaneously one hour before investigation in doses of 20 µg/100 g rat

ACTH was injected in a dose of 5 I U (Jaton prolongatum) one hour before sacrifice

Table I The basal content of phosphopyridine nucleotides in the liver in alloxan diabetes and after injection of insulin and adrenaline The rats had been fasted for 18 hours

0 1 unit insulin per 100 g rat was injected intravenously 1 hour before investigation. 20 μg adrenaline per 100 g rat was injected subcutaneously 1 hour before the rat was killed. The values represent μg per g liver

Experimental	No of rats	Content of DPN	Content of DPNH	Ratio DPN/DPNH	Content of TPN	Content of TPNH	Ratio TPN/TPNH
Controls	10	449 ± 10	145 ±	3 12 ± 0 08	83 ± 4	379 ± 10	0 22 ± 0 01
Alloxan diabetes	13	414 ± 19	162 ± 7	2 55 ± 0 06	82 ± 5	362 ± 15	0.23 ± 0 01
Controls	4	392 ± 10	133 ± 6	2 96 ± 0 13	55 ± 4	353 ± 27	0 16±0 016
Insulin injection	4	446 ± 18	110 ± 9	4 17 ± 0 33	52 ± 5	341 ± 6	0 15±0 017
Controls	6	396 ± 14	124 ± 10	3 30 ± 0.26	56 ± 4	353 ± 16	0 16 ± 0 01
Adrenaline injection	9	403 ± 16	109 ± [illegible]	3 71 ± 0 30	54 ± 1	345 ± 14	0 16 ± 0 01

Statistical significance of the differences

Alloxan diabetes versus controls DPN P 0 15 DPNH P 0 05 Ratio DPN/DPNH P 0 01
Insulin versus controls DPN P 0 07 DPNH P 0 01 Ratio DPN/DPNH P 0 01

TPN Lot 30 B-623 16 purity 98—100 /
DPNH Lot 80 B-705 purity 90 % TPNH Lot 30 B 634-3 purity 97
The preparations used for injection experiments were
Nicotinamide commercial preparation
Insulin Vitrum 40 units per ml Apothekernes Laboratorium Oslo
Insulin Retard Leo N I L *Protamin insulin crist 40 units per ml*
L-Adrenaline crist codex Rhone Poulenc Paris
L-Ethionine Sigma Chemical Co
ACTH (Jaton prolongatum) 20 units per ml Apothekernes Laboratorium *Oslo*

Results

The first results presented in Table I concern the basal level of the four types of pyridine nucleotides in liver extracts from control animals Also included are the values obtained with liver extracts from alloxan diabetic or insulin or adrenaline treated animals These animals were fasted for 18 hours before sacrifice to establish basal experimental conditions and to secure the optimal conditions for insulin action The effect of starvation on the content of pyridine nucleotides has been investigated by GLOCK and McLEAN (1955) and their results shall be shortly summarized before the present results are discussed These authors found that starvation for 24 hours did not decrease the total content of pyridine nucleotides A greater fraction however of the diphosphopyridine nucleotides was found in the reduced form An increase in the level of TPNH was likewise recorded while TPN showed very low values (10 μg/g) in their experiments both in fasted and fed conditions

Ncotinamide was d olved in saline and injected intraperitoneally 500 mg/kg Ethionine was lik wise dissolved in saline and njected intraperitoneally in a single dose of 150 mg per at of 300 g animal weight

Analytical pro edure Th preparat on of the liver extracts for the enzyme assay was performed acco ding to the method of C otti and KAPLAN (1957) Excision of the liver wa made immediately after the animals had been scarified by a blo v on the head and bled To avo d any spontaneous oxidation of the reduced pyrid ne nucleotides in the extract the ext a t v ere kept at pH 10 It is known that w th the aid of oxygen in the air and flavines in the extra t DPNH and TPNH are oxidized at neutrality to DPN and TPN respect ly (SINGER and KEARNEY 1950) At higher pH the reduced pyridin nucleot des are more stabl (KLINGENBERG and SLENCZKA 1959) The per cent reco ery during the ext act on p o edures as controll d by adding internal standard to the ext act on medium befo the tissue was added DPNH v as re covered with 107 / and TPNH v th 98 / (mean values)

In expe iments on the indu ed synthesis the extraction of the liver tissue for DPN det rmination v as done in 5 / TCA or n 5 / TCA + 0 1 N nicotinamide added Identical amounts of DPN e reco ded by the tv o proc dur s

The q antitativ determinations of the pyridine nucleotides er performed by the fluorometri meth d originally introdu ed by CARPENTER and KODICEK (1950) The det rminations v e e made on a Farrand pect ofluorometer With the exception of DPNH the enzyme as ay of JACOBSON and ASTRACHAN (1957) was used for the mic o det rmin tion of th pyridine nucl otides The fluores ent methyl ethyl ketone ad d tion p oduct was p epa d accord ng to CIOTTI and KAPLAN (1957)

Becaus of t DPNH pecific ty α glycero-phosphate dehydrogenase v as introduced in th enzyme assay (Holzer *et al* 1958) The rea tion mixture contained 0 5 ml of 0 05 M sodium ca bonate buffer pH 10 0 2 ml of 1M potass um phosphate buffer pH 7 5 0 01 *ml of tri e phosphate solution DPNH at various concentrations and 0 02 ml* of α glycerophosphate dehydrogenase Afte ncubation for 5 min at room t mperature 0 1 ml of 50 TCA vas added Fluo escense was dev loped by methyl ethyl ketone

In the determinat on of TPNH 0 1 ml of 2 × 10 M oxidized glutath one v as used tw ce the concentration of that r ported by JACOBSON and ASTRACHAN (1957)

Materials In the enzyme assay pro edures the foll wing enzyme p eparations vere us d Alcohol dehydrogenase f om yeast C F Boehringe & Soehne Mannh im The tock sol tion containing 100 mg in 3 4 ml v as d luted 1 40 before use with distilled v ate

I oc tric dehydrogenase S gma Chemical Co The p pa ation contained 14 or 20 R ck units/mg B fore use 3 mg was d ssol ed in 15 ml 1 5 M Tris-buff and 1 ml d t water

α Glyc ropho phate dehyd ogenase from muscle C F Boeh inger & Soehne The tock solution ont ined 10 mg/ml In the enzymat te t was used a solution ontaining *15 μl stock solution and 200 μl dist at r*

Glutathione r du tase S gma Chem cal Co 6 000 Racker un t /mg 1 mg was dis ol ed in 5 ml dist water bef e use

Th following substrate p eparations v re used

Trio e phosph te s lut on Na s lt of d hydroxya etone pho phate and glyce aldehyde phosphate C F Boehring & Soehne about 0 02 M of each

Glutath on S gma Chemical Co

Sod um iso-c trate S gma Chemical Co

Th pyrid n nucleotides used for the p eparat on of tandard curves and for o ery experiments, ere all products from S gma Chem cal Co

DPN from y a t Lot 40 B-627 7 purity 98

Table II Induced synthesis of DPN in the liver after injection of insulin

Nicotinamide was injected 12 hours prior to investigation. 0.1 unit insulin per 100 g rat had been injected intravenously 1 hour before investigation. Non fasted rats were used.

Experimental	Content of	
	DPN µg per g	DPNH µg per g
Controls	[illegible]5[illegible]0 ± [illegible]62 (7)	[illegible] ± [illegible] (4)
Insulin	2 120 ± 354 ([illegible])	[illegible] ± 16 (4)

( ) Number of animals.

Insulin versus controls DPN P > 0.2 *DPNH* P [illegible] 0.1

It may therefore be concluded that insulin introduces a small increase in the total content of diphosphopyridine nucleotides this effect being easier to demonstrate in fed animals which can draw upon their external supply for the biosynthesis No significant effect could be recorded in the basal level of pyridine nucleotides after injection of adrenaline

The induced synthesis of diphosphopyridine nucleotides was used in the present work for elucidating metabolic alterations due to hormone action. The results obtained are presented in Table II and III A group of non fasted [illegible]

Table III Induced synthesis of DPN in the liver after injection of [illegible] and ethionine

Insulin 0.1 unit per 100 g rat injected subcutaneously 1 hour before investigation
Adrenaline 0 µg 100 g injected subcutaneously 1 hour before investigation
ACTH 5 I.U. ([illegible] prolongatum) injected intraperitoneally 1 hour before in[illegible]
Ethionine 150 mg dissolved in 0.9 NaCl, injected 1 hour be[illegible] t was [illegible]
Nicotinamide was injected 1[illegible] hours before investigation [illegible] experiments. The rats [illegible] fasted for 18 hours.

Experimental	No. of animals	[illegible] mg per 1[illegible]
[illegible]	8	1[illegible]
[illegible]	8	[illegible]
[illegible]	4	1
[illegible]	4	[illegible]

[illegible] of the difference of DPN [illegible]
[illegible], P < 0.01 *ACTH versus contr[illegible]*

were injected intraperitoneally with nicotinamide 500 mg/kg rat weight which gives a maximal response as reported by KAPLAN *et al* (1956) The values given in Table II includes the level of diphosphopyridine nucleotides in rat liver extracts 12 hours after the injection of nicotinamide In accordance with the observations made by other investigators a high content of DPN was found In addition it was observed that some increase could be recorded in the reduced form but as mentioned earlier no effect could be observed in the triphosphopyridine nucleotides The injection of insulin did not significantly alter the level of the DPN or DPNH in these fed animals In fasted animals however a significant drop in the content of DPN was recorded as a result of insulin action In Table III the values for the pyridine nucleotide content in fasted controls showed a decrease compared to the fed control group Obviously the starvation was responsible for a restriction of the induced synthesis this being possible connected with a deficit in the adenine and ribose phosphate moieties the nicotinamide portion of the molecule being accessible in excess Under these conditions the injection of insulin introduced a metabolic alteration in the liver which resulted in a further drop in the content of DPN

Since the insulin injection affected the induced synthesis of DPN but was rather unreactive with respect to the basal content one had to assume a fundamental difference in reactivity between the DPN in the resting liver cells and the DPN originating from an external precursor The hypothesis was made that this last type could be regarded as a rather labile fraction without any other specialized basal function than that of supplementing other biochemical systems

As will be discussed below some regulatory mechanism of hormonal nature seems to control the level or induced DPN in the liver In the work of GREENGAARD *et al* (1961) on hypophysectomized animals the administration of ACTH (8 units daily for 6 days or one single injection of cortisone acetate 5 mg 30 min before injection of nicotinamide) completely normalized the high levels of DPN present in the livers of these hypophysectomized animals

It was found of interest to test the effect of ACTH on the induced synthesis of DPN in normal animals In the present experiments the ACTH was administered as one single dose at a time when a high level of DPN was present As shown in Table III a significant drop in the content was obtained The regulatory effect of ACTH was thus confirmed in normal animals To test the system with a substance which would interfere with the enzymatic DPN synthesis use was made of a single injection of ethionine As shown in Table III the injection of ethionine gave a lower net synthesis of induced DPN similar to the effect which has been described with azaserine

As the decrease in net synthesis obtained by insulin either could be attributed to an inhibition of biosynthesis or to an increased turnover of the DPN molecule the effect of insulin was recorded at different time intervals in the induced synthesis As shown in Fig 1 the level of DPN increased with time to a maximal value This was reached at an earlier stage under the experimental conditions

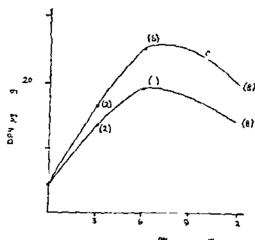


Fig 1 Time curve for the influence f insulin on the DPN content or rat li er after injection of nicotinamide All animals were injected intraperitoneally w th 500 mg nicotinamide per kg body we ght and killed after d fferent times. 0 1 unit insulin per 100 g rat was injected intravenously I hour befo e n est gation. Th e rats had been fasted for 18 hours. The curve represents m a values ( ) Number of animals.

used in the present work, than is reported by other workers for experiments with fed animals The insulin treated animals showed a lower content of DPN in the liver extracts at all time intervals investigated

It was found of interest to investigate the effect of insulin on the content of DPN in muscle tissue under nicotinamide loading Muscle tissue belongs to the group of tissues which do not respond with induced synthesis to nicotinamide injection in contrast to the group which readily responds (including liver dney spleen tumor tissue) In Table IV is recorded the level of DPN in the rat diaphragm 3 hours after nicotinamide injection Although the levels recorded were slightly higher than the basal values no inhibitory effect could be observed in the insulin treated group Preliminary experiments on adipose tissue seemed to indicate that this tissue also was rather insensitive toward nicotinamide injection and an insulin effect could not be obtained

The results reported by MIRSKY (1957) on the hyperglycemic action of nicotinamide prompted the investigations on the blood sugar level in the nicotinamide injected animals The results are presented in Fig 2 A fairly

Table IV The content f DPN in the diaphr gm fter jection f nicotinamide

N cotinam de and 0 1 u ns l n Retard per 100 g at w njected subcutaneously 3 hours bef r in est gation. The r ts had been fasted for 18 hours.

Co tent of DPN μg per g	
Control	Insulin
0 ± 4	545 ± 6

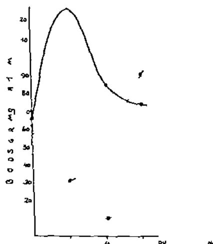


Fig 2 The hyperglycemic effect of nicotinamide in rats in the absence and the presence of insulin injection 250 g rats which had been fasted for 18 hours were used All injections were done at o tim

—— Injection of nicotinamide (500 mg per kg intraperitoneally)

... Subcutaneous injection of 0 25 units insulin. Ret d Le (Protamin insulin cryst.)

Nicotinamide and insulin injected simultaneously t time.

The curves represent average values from 4 rats in each group

rapid increase following the administration of nicotinamide was recorded In the insulin treated animals however no increase in blood sugar could be found The hypoglycemic response was present in the first two hours but in contrast to the hypoglycemic response in the insulin control group the length of the hypoglycemic period was shortened in the group of animals exposed to nicotinamide The blood sugar determinations were performed by the glucose oxidase method The mechanism by which the nicotinamide increases the blood sugar in fasted rats and shortens the hypoglycemic response of insulin is unknown More work is needed to evaluate any correlation between the nicotinamide induced DPN synthesis and the elevated blood sugar

Discussion

There seems to be a difference in reactivity of the pyridine nucleotides which are connected with the basal content in the resting liver cell and the pyridine nucleotides which are produced by the supply of an external precursor As is shown in the present work this last group normally shows great fluctuations and reacts more readily on hormonal and nutritional influences The basal content on the other hand showed only small variations even under conditions of severely impaired metabolism as in alloxan diabetes The only conditions which can severely affect the basal content are the treatment with azaserine NARROD *et al* (1961) have found a decrease in basal content in mouse liver DPN when azaserine was injected before sacrifice The values dropped from 445 μg/g liver to 143 μg/g The effect was reversed by the concomittant injection of nicotinamide Similar observations have been made by LANGAN *et al* (1959) These authors have investigated the diphosphopyridine nucleotides only As pointed out in the present work, the small alterations recorded

the basal level, concern the diphosphopyridine nucleotides only. Whether azaserine or compounds with similar effect, reduce the triphosphopyridine nucleotide content remains to be seen.

In the present work it has been recorded that the induced biosynthesis of the diphosphopyridine nucleotides can be affected by insulin and ACTH, as well as by ethionine, in fasted rat liver. In each case a decrease in net synthesis was recorded. The question then arised whether the active agents inhibited the synthesis or activated the normal turnover or whether the breakdown of DPN was accelerated. The synthesis of DPN requires besides the supply of nicotinamide, a mobilization or an increased synthesis of the other components in the DPN molecule. SHUSTER *et al.* (1958 a, 1958 b) have found that injection of nicotinamide gives a net increase in the total and soluble adenine, ribose and nicotinamide, but they found no detectable change in the amount of free adenine nucleotides as a result of this synthesis. The participation of the adenosine moiety in the induced synthesis has been discussed by the same authors, who investigated the turnover of [^{14}C] labelled ribose in liver after injection of nicotinamide. They found in fact a more rapid turnover in the adenine ribose after injection of nicotinamide than in the nicotinamide ribose. In the normal controls however the most rapid turnover was found in the nicotinamide ribose.

NARROD *et al.* (1961) have investigated the incorporation of the various moieties of DPN in the presence and absense of azaserine after nicotinamide injection. They concluded that azaserine produced a more rapid rate of destruction of the coenzyme compared to the rate of synthesis. Proof of this hypothesis was indicated by the observation that azaserine produced an increase in urinary secretion of nicotinamide metabolites. The DPNase was not affected by azaserine.

Enhancement of the turnover as well as the breakdown of induced DPN can be assumed to occur as a result of hormone injection. Inhibition of the normal turnover has been recorded by GREENGARD *et al.* (1961) in hypophysectomized animals. Pretreatment with ACTH or injection of cortisone acetate before the administration of nicotinamide prevented the extensive and longlasting increase in DPN which was recorded in the hypophysectomized animals.

In the present work it has been shown that the administration of insulin as well as ACTH to normal fasted animals lowers the elevated level of induced liver DPN. As these hormones exerted its action on the preformed elevated content of DPN in short time experiments one had to assume that the hormones acted on a rather rapid metabolic conversion process. Whether the hormones act by increasing the rate of DPN turnover or whether an activating effect is exerted on DPN-splitting enzymes, cannot be decided from the present work. This problem will be further investigated.

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Some Properties of the ATP Dependent Na^+ Binding System of Rat Brain Microsomes

By

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Abstract

JARNEFELT J and L. V VON STEDINGK *Some propert es of the ATP dependent Na bindin system of at b a n m rosomes* Acta physiol scand 1963 57 3 8—338 — The ATP dependent binding of Na^+ ions by microsomes isolated from rat bra n is described Data are presented on the stability of the bound sodium under arious conditions. The precipitat on of the microsomes at th end of the incubation with trong salt solutions stab lizes the bound sodium. The lowest blank values are obtained th NaCl as precipitat ng agent probably due to the isotope dilution effect Th precipitation with 1 M NaCl is adopted as the standard procedure Th bindin of sodium is dependent on the concentrations of ATP and of Na ions it is pecific for ATP as other nucleotides do not promote bind ing The binding s inhibited by 5 mM calcium Durin the condi tions of incubation sodium is bound reversibly and an exchange can therefore be observed In this exchange react on the rel ase of sodium from the particles appears to be inhib ted by 10^{-4} M ouabai

It is concluded that similarities between th described Na b nding system on one hand and active transport and th microsomal Na dependent ATPase on the other hand make t permissibl to assume that the binding of sodium ma represent part of a m echanism for the acti e transport of ons.

The difference in the concentration of sodium and potassium ions between the intracellular and the extracellular fluids is due to an energy requiring mechanism in the cell membrane capable of transferring the ions from a compartment with a lower electrochemical potential to another with a higher

The following abbreviations are used ATP CTP GTP and UTP the 5 u phosphates of adenosine, cytidine guanosin and uridine respect ly ADP aden . . . ATPase an enzymic activity caus g th h droly is of ATP to ADP and inorganic p . . . RNA ribonucleic acid Tris, tris-(h droxymethyl)- minomethane

potential That this process *i e* the active transport derives its energy from oxidative phosphorylation and more specifically from adenosine triphosphate or other high energy compounds has been shown by HODGKIN and KEYNES (1955) and by CALDWELL *et al* (1960)

The exact mechanism of the active transport is still quite obscure Recently it has been shown in several laboratories (SKOU 1957 1960 POST *et al* 1960 JARNEFELT 1960 1961 a 1962 DUNHAM and GLYNN 1961 DEUL and MCILWAIN 1961 BONTING *et al* 1961) that subcellular fractions mainly of the microsomal category contain an ATP hydrolyzing activity which is dependent on the presence of Na^+ and K^+ ions This ATPase shows many properties which indicate its close relationship to the process of active transport On the other hand the mode of involvement of Na^+ in this system has not been clarified During the homogenization of the tissue the essential property of the cell membrane of having an inside and an outside is lost and no net transport can of course be observed in systems consisting of membrane fragments only Conclusions can be drawn only by analogy and in the case of the ATPases by the fact that the hydrolysis of ATP is the most likely reaction to provide the energy for active transport Recently however it was possible to demonstrate an ATP dependent binding of Na^+ ions in microsomes isolated from rat brain (JARNEFELT 1961 b) The present communication reports in more detail some of the properties of the Na^+ binding The characteristics of the binding are not incompatible with the view that the bound sodium might represent the form in which it is transported through the membrane

Methods

Preparation of microsomes from rat brain

The microsomes were prepared as described earlier (JARNEFELT 1962) They were suspended in 0 25 M sucrose in a final volume of about 0 5 ml per brain This gave a protein content of about 10 mg/ml of suspension

Reagents

The reagents usually were of analytical grade Sodium and potassium salts were avoided when not intentionally added and where necessary converted to tris salts through the use of ion exchange resins ATP was obtained from Sigma Chemical Company as the crystalline Na salt and NaCl from the Radiochemical Centre Amersham England Ouabain was the product of Merck Darmstadt, and was obtained through the courtesy of Dr H Wallgren

Assay of Na binding

The binding of sodium to the microsomes was studied in the following incubation mixture with a total volume of 1 ml Tris HCl buffer pH 7 5 20 mM $MgCl_2$ 4mM NaCl 100 mM (except when specified otherwise) sucrose 175 mM and microsomes from about one rat brain (about 5 mg protein) ATP to a final concentration of 4 mM was added at 0-time after a preincubation of 24 seconds The experiments were carried out in Spinco no 40 lusteroid tubes at a temperature of 30 C with varying usually quite short incubation periods (3—120 sec) The reaction was stopped by the addi

Table I. Microsomal ATP dependent binding of Na. The blank value [illegible]20 counts/10 min was subtracted. Incubation time 12 sec

ATP added mM	Counts/10 min
0	[illegible]30
4	2,210

of 10 ml of an ice-cold solution, 1 M in NaCl, buffered with 20 mM tris HCl, pH 7.5. The microsomes were then sedimented in the Spinco model L centrifuge, rotor no. 40 at 105 000 g for 10 min. The pellet was washed once with 11 ml of 1 M NaCl-tris-HCl, resedimented, and the radioactivity remaining in the pellet was measured in a well scintillation counter.

The NaCl in the incubation mixture contained about 1 μC of ^{24}NaCl, giving about $14-17 \times 10^4$ counts per 10 min.

Determination of Protein

Protein in the original microsome suspension or in the final pellets was determined by the biuret reaction using the procedure of GORNALL *et al.* (1949). The suspension was clarified with deoxycholate.

Results

The binding of Na^+ by microsomes

Table I illustrates the ATP dependence of the binding of Na^+ by brain microsomes. Usually a 4 to 6 fold increase in the initial sodium incorporation rate is seen in the presence of ATP. In the experiment shown, the blank binding has been subtracted.

In order to understand the binding phenomenon better it is of some interest to obtain information on the stability of the bound sodium. As indicated the reaction was terminated by the addition of ice cold 1 M NaCl solution. The reason why this stops the reaction could partly be due to the chilling of the solution, partly the high salt concentration may be the cause. To investigate the latter point varying concentrations of NaCl were used to stop the reaction as well as other salts, such as KCl and ammonium sulfate. Table II shows some results. The amount of sodium bound to the pellet in absence of ATP does not vary very much with varying concentrations of NaCl, whereas about twice as much is found when 1 M KCl or 1 M ammonium sulfate is used. The increased blank binding is probably due to the absence of an isotope dilution effect. The excess Na bound in the experiments where ATP was present increases slightly with increasing Na concentration in the washing medium. When KCl is used instead of NaCl, an equal ATP dependent binding is observed but in the experiment with ammonium sulfate the amount of bound Na is only about half. Many experiments of the type related above and others have given good reason to believe that the blank binding is not due to true binding

T bl II Amourt of dioact ity found in pellet usin various lt media for stopping the react on The total radioactivity is given no 0 time values ha e been subtracted Counts/10 min Incubation t me 6 sec in pr sence of ATP

	Washing m dium		ATP independent counts	ATP dependent counts
Experiment 1	N Cl	0 5 M	? 634	1,201
	N Cl	1 0 M	2 090	1,564
	N Cl	? 0 M	2 131	1 ,6?
	N Cl	3 0 M	2 401	1 881
Experiment ?	KCl	1 0 M	5 612	1 696
	Ammonium sulfate	1 0 M	4 115	813

T ble III The amount f ATP dependent rad oactu ty in pellets obtained with v rious pH values n the st pping medium Incub tion time 6 The values e r unded ff means f 2 to 4 sep ate leterminat ons

pH of washing med um (1 M N Cl)	ATP dep dent unts
4 0	700
4 5	1 400
5 5	3,200
6 0	2 800
7 5	3 000
8.5	2 000

of sodium to the microsomes but rather represents mechanical contamination by the suspension medium

If trichloroacetic acid perchloric acid or dilution with sucrose were used to stop the reaction no ATP dependent binding was observed

In Table III the effect of variation of the pH of the 1 M NaCl added to stop the reaction is shown Between pH 5 5 and 7 5 a rather constant binding is seen At the extreme pH values some decline in the ATP dependent binding is evident

The determination of the protein recovered in the final pellets indicates that about 60—70 per cent of the protein originally added is precipitated in the procedure used Within one series of experiments the recovery is very constant showing a variation in the different tubes of not more than 1—2 per cent The addition of the various chemicals used in the experiments does not alter this recovery It seems fairly safe to assume that the same fraction qualitatively and quantitatively is always recovered and that the variations seen in

Table IV Dependence of Na^{+} binding on the concentration of ATP Incubation time 6 sec

	ATP mM	Counts/10 min /mg
Experiment 1	0	123
	1 0	137
	1 6	202
	2 0	304
	2 7	413
	4 0	416
Experiment 2	4 0	372
	8.0	353
	12 0	377

sodium binding express variations in the reactivity of this fraction towards sodium

Dependence on the concentration of ATP

The amount of bound sodium in the pellet is dependent on the ATP concentration of the reaction medium (Table IV) A certain threshold concentration of ATP seems to exist since below 1 mM ATP very little binding is seen At a 3—4 mM concentration maximal binding is obtained Partly because of this peculiarity of the ATP dependence partly because of the very high ATPase of the microsomes the determination of the usual kinetic constants has not been attempted

Dependence on the concentration of Na

Fig 1 illustrates that the binding of Na is highly dependent on the concentration of Na^{+} in the incubation medium Since it was established that high concentrations of NaCl terminate the reaction it is not surprising that a slight decrease in the binding rate is observed at concentrations above 0 1 M Again this induces difficulties in the determination of kinetic constants for the reaction From the double reciprocal plot shown in Fig 2 a K_m of 0 1 M Na is obtained and we think that this value is meaningful

Nucleotide specificity

Only ATP of the nucleoside triphosphates is able to increase the binding of Na^{+} by the microsomes Some data on the specificity are collected in Table V The ATP specificity is in good agreement with observations on the Na dependent ATPase of the microsomes since an increase in the activity is caused by Na^{+} only when ATP is the substrate even though the other nucleoside triphosphatases *per se* are about equally active No activity is seen with ADP in the Na^{+} binding assay Addition of other nucleotides together with ATP did not alter the activity obtained with the latter alone

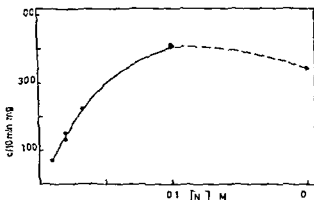


Fig 1 Dependence of sodium incorporation on the concentration of sodium in the incubation medium Incubation time 6 seconds.

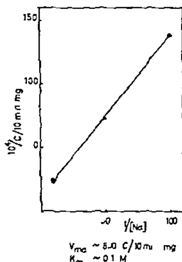


Fig 2 Lineweaver — Burke plot of the data shown in Fig 1

Inhibition by calcium

The binding of sodium is strongly inhibited by the presence of calcium in the incubation mixture (Table VI) It is to be noted that Ca^{++} is known to inhibit the active transport of sodium and that it also inhibits the Na dependent ATPase of brain microsomes (JARNEFELT 1962)

The stability of bound Na during the conditions of incubation

From the standpoint of the mechanism of the binding of sodium and its relation to the active transport it is of some importance to establish whether the sodium has been bound irreversibly or whether it can be actively in an ATP dependent manner released again In the latter case it should be possible to observe an exchange reaction Attempts to do this have been made

Table V The nucleotide specificity of the binding of sodium by microsomes Incubation time 6 se… The appropriate blank values were subtracted

	Substrate	Concentration mM	Count/10 min×mg
Experiment 1	None	—	75
	ATP	4.0	353 (+278)
	CTP	6.8	153 (+ 78)
	CTP	7.8	80 (+ 5)
	UTP	7.0	107 (+ 32)
Experiment 2	None	—	178
	ATP	4.0	428 (+250)
	CTP	3.4	151 (− 27)
Experiment 3	None	—	119
	ATP	4.0	365 (+246)
	ADP	4.0	109 (− 10)

Table VI The effect of calcium on the binding of sodium by microsomes Incubation time 12 sec

	Count/10 min	
	Exp 1	Exp 2
No ATP	387	731
4 mM ATP	2 460	2 847
4 mM ATP + 5 mM Ca	724	683

abruptly altering the specific radioactivity of the sodium in the incubation medium and observing the amount of radioactivity in the final pellet after an additional incubation period Table VII contains the data on two experiments In the first the specific radioactivity was reduced to one third by dilution with a twofold volume of a solution containing 0.1 M nonradioactive NaCl and in the other the specific activity was increased twofold through the addition of more NaCl in a very small amount of total NaCl Both types of experiments indicate that a much more pronounced change in bound sodium occurs if ATP is included in the added solution It was established in separate experiments that at the moment of this addition usually one minute after the initiation of the experiment only negligible amounts of the originally added ATP remained To us the ATP dependence of the sodium binding in this kind of experiment indicates an exchange type of binding where the bound sodium ion is released and immediately replaced by another The exact description of this exchange must await further experimentation since in addition to it changes in the total amount of bound sodium seem to take place when the assay mixture is diluted and when ATP is added to it

Table VII The ff t of a sudden hang f the sp fic radi act v ty of the ²²Na in the ncuba ion medium In experiment 1 afte a p ncubation in p sence of ATP the med um was diluted decr asin the pec fic act v ty t ne third of the riginal In xperiment 2 mo ²²NaCl was added t ncr ase the p f c act v ty to tw e the origin l alue Note espe ally the 3-m n d v lues

	ATP	Rad oact vity of p ll t counts/10 m n			
		At 60	At the nd of an add t onal		
			3	12	60
Experim nt 1	+	9 757	6 531 (—3,226)	6,549 (—3,208)	—
(dilut n f Na)	—	9 757	7 887 (—1 875)	7 090 (—2 667)	—
Exper ment 2	+	12 39	17 629 (+5,234)	18 103 (+5 08)	21,246 (+8 8 1)
(addition of Na)	—	12 395	14 674 (+2,2 9)	17 472 (+4 0 7)	20 532 (+8 137)

Inhibition by Ouabain

In ordinary binding experiments the effect of ouabain was to increase slightly the amount of bound sodium However as illustrated in Table VIII in an experiment made to observe the exchange of bound sodium by decreasing the specific radioactivity a pronounced inhibition was seen in the presence of ouabain This result indicates that the release rather than the binding of sodium is inhibited by ouabain Such a behavior would be in remarkable agreement with the concept that the site of action of ouabain is on the outside of the cell membrane (CALDWELL and KEYNES 1959)

Comparison of the incorporation of Na with that of ³²P from terminally labelled ATP

Since there are indications that some phosphate containing compounds i e phosphatidic acids (HOKIN and HOKIN 1960) or phosphoproteins (HEALD 1962 AHMED and JUDAH 1962) may be involved as carriers in the active transport of sodium a comparison of the Na and phosphate incorporations was made Table IX shows the results of such an experiment where terminally labelled ATP was used as substrate Very much less phosphate is incorporated during all the intervals tested and it is clear that intermediates based on this phosphate could not explain all the bound sodium

Discussion

The data related above indicate that an ATP dependent binding of sodium takes place in isolated microsomes from rat brain The general significance of this observation must be discussed from several points of view First it would be important to establish the identity of the cell fraction studied in terms of organelles of the inta t cell Earlier work (JARNEFELT 1962) seems to rule out mitochondria as a major constituent of this fraction The low centrifugal

Table VIII *The effect of ouabain on the ATP dependent binding (exp 1) and the exchange (exp 2) of Na by microsomes from brain In the exchange experiments the specific radioactivity of the Na was reduced to one third by addition of nonradioactive Na after a 60 second preincubation The reaction was then terminated in the usual way at 63 sec*

	Count /10 min
Exp 1	
Binding of Na in presence of ATP	
No ouabain added 12 sec	1 7[illegible]6
10^{-5} M oubain present	1 [illegible] 6
Exp 2	
Exchange of bound Na^+ in presence of ATP[1]	
Present at 60 sec	7,273
+3 sec., no ouabain	4 689 (—2 [illegible]84)
+3 sec 0 7 × 10^{-5} M ouabain	5 477 (—1 [illegible]96)

[1] Compare this experiment with exp 1 in Table VII

Table IX *Comparison of the incorporation of ^{22}Na and of the terminal phosphate (^{32}P) of ATP into microsomes*

Incubation time sec	^{22}Na incorporated, mμmoles/mg		^{32}P incorporated mμmoles/mg
	No ATP	4 mM ATP	
12		2 [illegible]	0 04
[illegible]0	0 8	4.3	0 11
[illegible]0	0.8	[illegible].2	0 59

used in the sedimentation of the fraction in question speaks on the other hand against the possibility that it would contain mainly ribosomes In fact the RNA to protein ratio of this fraction is much lower than that of the fraction sedimented at high speed from the supernatant (JARNEFELT 1962) Thus it seems likely that mainly membranous structures of the microsomal fraction are found in the material studied This view is strengthened by the work of HANZON and TOSCHI (19[illegible]9) where it was shown that mainly membranous structures were sedimented at centrifugal forces used in this investigation The question of how much of this material originates from the outer membrane of the cell and how much is derived from the endoplasmic reticulum has to remain open so far

If the sodium binding is assumed to be a part of the active transport of sodium across cell membranes it is important that it shows properties that are compatible with known properties of active transport. Both processes require ATP and both are inhibited by calcium Also the fact that the binding of sodium seems to be due to an exchange reaction with continuous uptake and

release of sodium can be taken as an indication that it could be involved in active transport. This process would of course just require such a mechanism. An indicative feature is also the inhibition of active transport by ouabain and the partial inhibition of the exchange of bound sodium by this compound.

Another question of interest is the relation of the sodium binding reaction to the Na^+ and K^+ stimulated ATPase of brain microsomes. The two reactions show a number of common features. The properties of the sodium stimulated ATPase have been studied extensively by several investigators and in several different tissues (SKOU 1957, 1960; POST *et al.* 1960; JARNEFELT 1960, 1961 a, 1962; DUNHAM and GLYNN 1961; DEUL and MCILWAIN 1961; BONTING *et al.* 1961) and it is generally agreed that this ATPase may reflect the active transport mechanism. The finding of the ability of the microsomes to bind sodium seems to strengthen this view as it in a way provides the missing link: the actual participation of sodium. The possibility to demonstrate a binding of sodium to the membrane fragments gives additional advantages to the system since the transient binding of sodium is a common assumption in most hypotheses dealing with the mechanism of active transport.

It has not been possible to obtain any clues as to the nature of the site to which sodium is bound. From data on the turnover rates of some phosphate containing substances found in the membranes HOKIN and HOKIN (1960), HEALD (1962) and AHMED and JUDAH (1962) have drawn the conclusion that such substances might function as carriers in the active transport of sodium. The experiment illustrated in table IX does not show any clear and simple correlation between the amounts of phosphate and sodium incorporated. The bound sodium could therefore hardly all be bound to a phosphate containing carrier. On the other hand it must be remembered that all the sodium does not necessarily have to be bound to the enzyme or carrier site. The microsomes when isolated usually appear in the form of small vesicles and it is possible that the sodium is accumulated inside these vesicles without being bound to a specific site. The question whether sodium is collected in this way inside a vesicle or whether it is bound to a specific site has not been resolved but work to decide the question has been planned. Before a successful experiment on these lines has been done it is not possible to make a fair comparison between the data for phosphate and for sodium incorporation.

HASSELBACH and MAKINOSE (1961, 1962) have described an ATP dependent Ca^{++} binding system occurring in the relaxing factor preparation of muscle. This system resembles in many respects the one described in the present communication. It would indeed be interesting to see whether the relaxing factor preparation also contains a Na^+ binding system since its ATPase is dependent on the presence of Na (JARNEFELT 1962).

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Acta physiol scand. 1963 57 339—351

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The Correlation between the Occurrence and Localization of Acetylcholinesterase rich Cell Bodies in the Stellate Ganglion and the Outflow of Cholinergic Sweat Secretory Fibres to the Fore Paw of the Cat

By

FOLKE SJOQVIST

Rece ed 17 August 196

Abstract

SJOQVIST F *The c rrel t n b tw en the o currence and lo ali al o f acetyl holine teras rich ll bodi s in the stellat g n lion and the o tfl u of hol ne gic ueat secr tory fibr s to the fore paw of the cat Acta physiol cand* 1963 57 3 9—351 — The per pheral a eas of innervation ha e been determin d for tho e pe al neu ons in the stellate ganglion which resemble histochem cally establi hed cholinergic nerve cells (type I cells) The approa h employed is based on the f ct that axotomized ganglio i c lls lose most of their AcChE and dent ty in sta ned ect ons A series of axotomies on diff rent postganglion c ami sho ed that the tellate ganglion s composed of d ffe ent zones w th highly varying amounts of type I cells Pra tically all of t type I c lls send th ir axons a th tebral nerv an l the 1st tho a c grey ramus to nervate the fore limb whe eas their axons seem to b ab ent from t e card ac n rves. H gh AcChE activ ty is found in th nerve terminals around the eccrine sw eat glands f the pa v and n som of the axons running in the po tgangli ni ram to the fore limb Fin lly a d t correspondence ha be n demonstrated betw en th number of t pe I-c lls in certain ones of the ganglion and the sw eat secretory esponses on the paw The esults suppo t th h pothesi that the type I-cells n the stellat gangl on of the cat a cholinerg c and innervate the eccrine weat glands

A few sympathetic ganglion cells possess outstandingly high acetylcholin esterase activity (AcChE) as demonstrated both histochemically and biochemically (KOELLE 1951 1955 HOLMSTEDT and SJOQVIST 1959 GIACOBINI 1959 HOLMSTEDT LUNDGREN and SJOQVIST 1962 FREDRICSSON and SJOQVIST 1962) In this respect these neurons resemble the cholinergic cells of the anterior

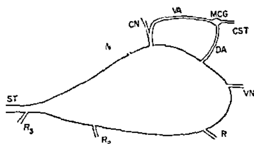


Fig. 1. Gross anatomical arrangement of the left stellate ganglion in the cat.

ST thoracic sympathetic trunk; CST cervical sympathetic trunk; MCG middle cervical ganglion; R1—R3 mixed grey and white rami of spinal nerves ThI—ThIII; DA and VA dorsal and ventral limb of *ansa subclavia* (preganglionic fibres to the superior cervical ganglion); CN cardiac nerves; VN vertebral nerve (grey rami to spinal nerves CIII—CVIII). The sympathetic outflow to the fore limb originates from ThI—CVI (brachial plexus).

horn and parasympathetic ganglia. Consequently these particular sympathetic neurons have been postulated to be cholinergic but direct proof for this hypothesis has up till now been lacking.

An accumulation of heavily stained or AcChE rich nerve cells is found especially in the stellate ganglion (HOLMSTEDT and SJÖQVIST 1959) and the purpose of the present investigation has been to associate these cells with their appropriate postganglionic outflow and if possible link them to some specific function. The approach to this study is based on the fact that axotomized ganglion cells lose most of their AcChE (BROWN 1958, FREDRICSSON and SJÖQVIST 1962). Thus the various postganglionic rami of the stellate ganglion have been sectioned and the ganglion studied histochemically with respect to its loss of heavily stained cell bodies.

Material and Methods

1. Anatomy of the stellate ganglion

Fig. 1 showing the principle arrangement of the left stellate ganglion is based on dissections of more than 40 cats. It is essentially in agreement with similar figures by LANGLEY (1893), BLIN (1958) and HOLMES and TORRANCE (1959). The anatomy of the cardiac nerves (CN) is not constant since they may arise either directly from the ganglion or from the ventral limb of *ansa subclavia* (VA). Generally one or two branches are seen. The supply of grey rami to the cervical spinal nerves (CIII—CVIII) varies. Generally it emerges from the stellate ganglion as a common nerve bundle (VN) which gives off separate strands to CVII and CVIII as well as the genuine vertebral ramus to CIII—CVI. For the sake of simplicity the composite nerve bundle of rami has been denoted vertebral nerve (VN) in accordance with the nomenclature used by some other authors (HOLMES and TORRANCE 1959). The operative procedure was adapted always in accordance with the anatomical variations.

As may be seen in Fig. 1 the cardiac and vertebral nerves are [illegible] postganglionic, whereas the rami to the thoracic spinal nerves contain both pre- and postganglionic fibres which are impossible to separate selectively. The white rami from ThI—ThIII terminate mostly in the superior cervical ganglion while the predominant preganglionic supply to the stellate ganglion is conveyed by way of the thoracic sympathetic trunk (LANGLEY 1893). Transection of the mixed rami R1—R3 therefore produces an axotomy of neurons located in the stellate ganglion in addition to a major denervation of the superior cervical ganglion, the latter being of no consequence to the present study.

B Operat ve procedu es

Cats v eigh ng between 1 8 and 4 2 kg were used They were an-esthetized v ith sodium pentobarbital (Nembutal Abbott, 40 mg/kg i p) and maintained in an ade quate depth by injecting through a cannula inserted into an extremity ve n Artif cial respiration was performed w th a tracheal intubation tube (Kifa Swed n no 2) con nected to a resp ration pump

The stellate ganglion was approached by an open thoracic procedure An incision v as made in the skin between the f rst and second rib The outer layer of intercostal muscles was divided along the ent re length of the surgical field The innermost layer was cut through near the sternal bone and a hole 2 cm in diameter produced which op ned into the thoracic ca ity

The different po tganglionic rami then we e dentif ed eas ly and transected about 1 cm from the ganglion body As much as possible of the distal ends v ere resect d The hole in the chest was closed during cont nuous suction of the pleural cavity and while the lung was inflated The wound was sutu ed compl tely and treated in accordance v ith a cepted surgical princ ples All cats we e administ red routinely 1 ml Str ptopenin (Kab Stockholm) and f necessary parent ral fluid therapy

Th fun tional derangements produced by the operations were followed on a number of peripheral sympathetically innerv at d organs Sectioning of R1—R2 resulted in different degrees of Horner s syndrome sev erance of the ve tebral nerv e and R1 caused anhidrosis and an ncr ased temperature of the fo e paw tc The symptoms also served as a check on the occurrence of functional regene ation v hich for the most part was negl gible

The cats w re sacr f ced ?—3 weeks aft r axotomy at v hich time the operated ganglion v as dissected out together w th the contralateral on The denervation experiments are summar zed n Table I

C Ply i l gic l nt l of pe at ons

The influenc of the diff ent ope ations on the sweat s cr tion in the fore paw was studied in six normal and six operated cats Supramaximal stimulation v as obta ned with a Grass st mulato model S4 and shielded s lve elect odes placed on the stellate ganglion or its different postganglionic arm The stimuli we e applied during a pe iod of 1—2 m n v ith a f equency of 5—15/ ec a durat on of 5 msec and ntensity of 4V

The sv eat cr tory respon es re studied both w th the naked ye and with the star h odm r action acco ding to RANDALL (1946) Th pads er paint d v ith a 3 odine olut on and allow d to dry A starch paper was pressed lightly against the paw during the last 15 s c of the timulation period Th result ng bla king of the sta ch paper ndi ated th d gre of weat ng and the p oport onal paw urface nvolved Control pe iods thout st mulat on of the ganglion we e included

D Histochem l p dur

The diss ted gangl a wer frozen mmed at ly in isopentane hilled with l qu d nitrog n and k pt at — 0 C f r ?—24 hours Cryostat erial s ctions (DITTE HEIDELBERG) 7 and 10 μ thi k wer p epared and stained alternat vely for Nissl substance (t lu dine blu) and AcChE a ord ng to HOLMSTEDT (1957) The ncubation per od v as held out nely t 0 m n but n a fev xperiments p olonged to 1—2 hours The AcChE- tain d ctions we used fo quant tative and qual tative tud es of the AcChE-contain ng gangl on c lls Th toluidin blue tain d p eparations revealed the degr e and ext nt of the h omatol sis tl er by v al ng the zones that ga e rise to the sectioned axon (FREDRIKSSON and SJOQVIST 1962) In some experim nts sect ons of the pads of th pa s wer also ta ned fo hol neste as

Table I. Types and number of nerve transection experiments on the stellate ganglion

Type of transection (Branches cut)	Number of ganglia investigated for type I-cells				Total
	Quantitatively		Qualitatively		
	Left	Right	Left	Right	
Normal (control)	8	5	13	16	42 (25)
Cardiac nerves	1	—	2	—	3
All branches except for cardiac nerves	2	—	1	—	3
Vertebral nerve	2	—	2	—	4
All branches except for vertebral nerve	1	—	—	—	1
R1 + R2	—	—	1	—	1
Vertebral nerve + R1	2	2	—	—	4
Total	16	7	19	16	58 (41)
	23 (16)		35 (25)		

The number of cats is given within parenthesis when not identical with the number of ganglia.

E. Biochemical procedure

In conjunction with the histochemical investigation on the ChE of the sweat glands of the pads a biochemical control of enzyme specificity was carried out. The pads of the paws were excised, freed from the outer skin layer, homogenized and determined for ChE titrigraphically according to HOLMSTEDT, LUNDGREN and SJÖQVIST (1962). The substrates employed were acetyl beta methylcholine iodide 6.0×10^{-3} M and butyryl choline iodide 6.47×10^{-3} M in final solutions.

In order to obtain reasonable activity it was necessary to use pads from all four feet. Extraneous enzymic activity resulting from sequestered red cell AcChE in the tissue was minimized in experiments repeated on paws that had been perfused for 2 hours with a physiological saline solution.

F. Quantitative determination of heavily stained neurons in different zones of the stellate ganglion

Two cell types of sympathetic ganglia show definite histochemical staining for AcChE, the type I-cells exhibiting heavy staining intensity and the type II-cells showing moderate degree of staining (for definition of cell types see FREDRICSSON and SJÖQVIST 1962).

Quantitative counts of type I-cells were performed partly on the operated ganglia in which remaining type I-cells in the non-chromatolytic zones were determined. The different operations served as controls of one another. Some frequency diagrams were obtained also from normal ganglia. In such cases, the different zones of demarcation were defined in accordance with the findings reached in the denervation experiments and were in general distinguished easily, especially in sections containing the various postganglionic rami.

All cell counts were made on serial sections because of differences in the organization of the ganglion at various depths. Initially the total number of neurons in a section was determined in a counting chamber and subsequently the type I-cells were counted separately, their frequency being expressed in per cent of the former. Approximately 2 000 cells per ganglion were counted. A total of 23 ganglia from 16 cats were investigated quantitatively and in addition 35 ganglia from 25 cats qualitatively (see Table I).

Results

I Experiments on induced retrograde changes in stellate ganglion

a Transection of cardiac nerves

Cutting of the cardiac nerves resulted in a chromatolysis of ganglion cells in the ventromedial part of the stellate ganglion *i e* the cardiac zone (Fig 2 bottom) Although scattered chromatolytic cells were seen also in other parts the heavily stained neurons (type I cells) occurred in the same number as in the control ganglia Thus it became apparent that the cardiac zone corresponded to that part of the ganglion which was virtually free of type I cells (Fig 2 top) On the other hand a high frequency of type I cells was found in the rostral and caudal poles of the lateral zone.

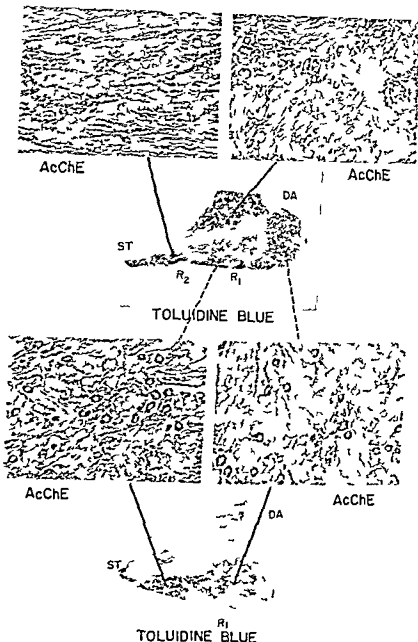
b Transection of all stellate branches except for cardiac nerves

Severance of all emerging nerves with exclusion of the cardiac branches produced chromatolysis of the whole ganglion except for the cardiac zone Only infrequently were normal cells seen in the chromatolysed parts No type I cells were in evidence in the ganglion including the cardiac zone. This indicated that the occasional type I cells that are localized anatomically within the cardiac zone do not send their axons to the cardiac nerves

c Transection of grey rami to spinal nerves performed in different combinations

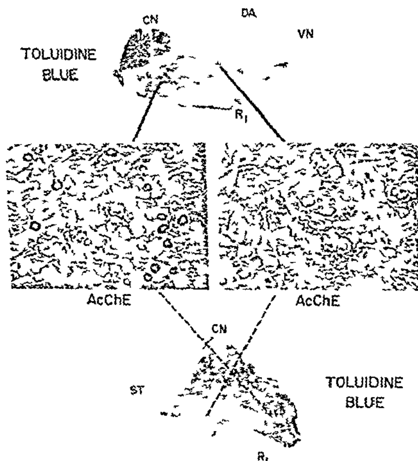
As an inspection of Fig 3—4 reveals practically all type I cells were found to send their axons through the vertebral nerve and first thoracic grey ramus When these two nerves were cut (Fig 4) only occasional heavily stained cells remained Furthermore resection of the vertebral nerve above CVI was without effect on the frequency of type I cells Evidently these cell bodies send their axons through the brachial plexus to innervate the fore limb (see Fig 1)

The different zones of the stellate ganglion are easily distinguished in Fig 3—4 The vertebral zone is the largest one followed by the cardiac zone the latter is somewhat larger on the right side than on the left Further subdivision of the vertebral zone into its constituent cervical components was not performed It was evident however that its most cranial and medial parts contained very few if any type I cells The basis for this paucity in type I cells very likely lies in the fact that this region does not supply fibres to the brachial plexus Although comparatively small the zone giving rise to R1 shows the greatest number of type I cells on a relative and sometimes on an absolute basis This is especially true for its central part where a cholinergic nucleus containing more than 30 / type I cells may be seen The zone constituting R2—R3 is extremely small Consequently its absolute number of type I cells is inconsiderable (see Fig 5)



Fi[illegible] 2. Top [illegible]ormal ganglion d[illegible]ided [illegible] [illegible]edial part [illegible] cardiac zone ([illegible]per [illegible]) a[illegible]d lateral part (low[illegible]) by th[illegible] main mass [illegible] p[illegible]ega[illegible] [illegible] ST—DA) [illegible] the cervical [illegible] thetic trunk. No type I-cell[illegible] ar[illegible] seen in th[illegible] ca[illegible]dia[illegible] [illegible]ght and only one in the most caudal part giving [illegible]ise to R[illegible] [illegible] Th[illegible] lateral part [illegible]o[illegible]tains a h[illegible]gh f[illegible]eq[illegible]ncy of type I-cells. H[illegible] stained preganglionic f[illegible]bres ar[illegible] seen everywher[illegible]

Bottom Cardiac nerves cu[illegible] Cardiac [illegible]on[illegible] [illegible]sually [illegible]mall Th[illegible] h[illegible]gh frequency [illegible] type I-cells in the lateral part of th[illegible] ganglion is unaffected by the [illegible]pe[illegible]

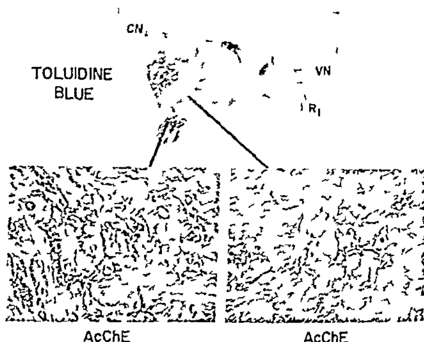


F g 3 T p \ rt b al n rv ut Loss f t pe I-cells fr m h axotom ed rt b al zon but till a h gh f equen ma n ng n th n f R1
Bott m Th am (R1—R cut Con rse esults f om p eding ope at n

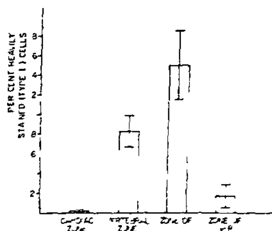
Gen ral leg nd to F g 2—4

Th tolud n l l t ined t ons l w h hol gar gl n w h is diff ent rami nd cated a co ding to F g 1 M n m gr ficat n 10 × Observ th faint taining in th axot mized z nes (urv l m ks wh h h gher m gnif sh w all gns of chromat lysis (se Fg 13 in FREDRICS o and Sjo ist 196

Th AcChE-p tures d m nst t h occurr f h a ly tained eur ns (type I-c lls) in diff ent nes f th g gl A t u u l n is dr wn o th p rt ph tom crogr ph d A d tt d lin gnif es th t th AChE p t is p ese ta f that particular gangl n n al h gh t is tak n from th h gangl — Cr ta sec ons Incubation im 30 min Phase-cont ast m cros py M gn f a n 80 All ph om gr phs w taken with th sam l ght ntens t a d pos re t m and wer t l d



Fi~ 4 Vertebral nerve and R1 cut. Observ the f rmat n of e r ma at the s t f operation. R latı ely larg cardiac zon Non or only ngl type I-cells may be present in the zo e of R...



F Frequency hist gram f type I-cells in the d ffer nt zones f the tellat ga gl on Observe the d ...er er ces betw n th for leg" zones and the cardiac part (see page 5 Th f gures ep esent m eans ± S.D The m an n ber of counted ... per zo and ar d m was (...r f experiment w than paret hes Cardia 00 (1 ertebral 00 (13 R1 00 (14 R —R3 00 (9

II Frequency of type I cells in differe t parts f the stellate gang lion

As shown in Fig 5 type I cells occur in highly variable amounts in the different zones of the stellate ganglion There are practically none in the cardiac zone and only a fe in the zone c rresponding t the lower th racic rami (R2—R3) In contrast to these zones f l v incidence there are higher frequencies in the zone f the first thoracic grey ramus (15 %) and in the vertebral zone (8)

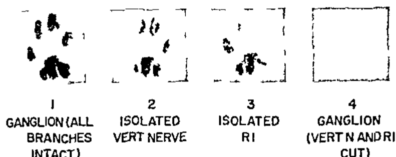


Fig 6 In spite of the great differences in si b tween th vert bral and R1 ones (se Fig 3) th xt ns on and d gree of sweating foll wing stimulat on of the rresponding rami are mo t often qu alent. Th explained by the cons derably higher inciden e f type I-c lls in th smaller R1 n The absol t numbers f such cells w thin thes t o zones are theref usually of the same ord r of ma nitud

III Sweat s cretion in th fore paw as related to the occurrence of type I cells

The only rami of the stellate ganglion that evoked sweat secretion on the pa when stimulated electrically were R1 and vertebral nerve (Fig 6) When marked divergences in content of type I cells occurred between the R1 and vertebral zones corresponding differences were observed between the secretory responses Following selective axotomy of R1 or VN a direct correspondence was in evidence between the number of type I cells remaining in the ganglion and the extension and degree of sweating on the paw after stimulation

IV Histochemical and biochemical investigation of the cholinesterase in the sweat glands of the fore paw

Histochemical staining of the paws showed considerable AcChE activity in structures suggestive of nerve terminals around the sweat glands (Fig 7) After sectioning the vertebral nerve and the 1st thoracic grey ramus there was a decrease in AcChE activity of the inner and outer parts of the paw respectively Low amounts of AcChE remained after the combined operation

Homogenates of non perfused and perfused pads of the paw hydrolysed Mecholyl a substrate pecific for AcChE The activity of the pad enzyme was investigated in the presence of other substrates and selective inhibitors for AcChE and BuChE The experiments showed that the activity was predominantly due to AcChE

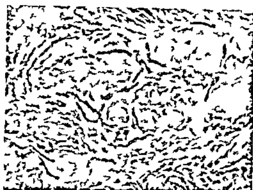


Fig. 7. Histochemical staining of AcChE in the sweat glands of the paws. Cryostat sections. Incubation time 30 minutes. Phase-contrast microscopy. Magnification 240 ×

Discussion

The purpose of the present study has been to seek an explanation for the high frequency of heavily stained AcChE-rich neurons in the stellate ganglion relative to some other ganglia of the cat (HOLMSTEDT and SJOQVIST 1959). It has previously been proposed that the heavily stained cell bodies (i. e. type I cells) give rise to postganglionic cholinergic fibres because of their histochemical resemblance to established cholinergic cells elsewhere (KOELLE 1951—1962, HOLMSTEDT and SJOQVIST 1959, GIACOBINI 1959).

In order to throw light upon the function of these cells an attempt was made to elucidate their innervation area. The approach employed was based on the fact that AcChE activity of sympathetic ganglion cells decreases markedly after axotomy resulting in a loss of staining (BROWN 1958, FREDRICSSON and SJOQVIST 1962).

Practically all the axons of type I cells were found to innervate the fore limb. The occasional type I-cells demonstrated in the cardiac zone remained after the cardiac nerves were cut. Their location near the limbs of *ansa subclavia* and their disappearance after sectioning of these rami make it probable that their axons follow the cervical sympathetic trunk rostrad. An organization of this kind may also account for the finding of scattered type I cells within the preganglionic cervical sympathetic fibres adjacent to the middle cervical ganglion (FREDRICSSON and SJOQVIST 1962). Postganglionic fibres from this ganglion communicate sometimes with lower cervical spinal nerves (LOLIH 1875).

Besides having their cell bodies in the stellate ganglion and being confined to the limb, the nerve terminals of the presumed effector organ may be expected to possess reasonable AcChE activity, since this enzyme generally occurs in approximately equal amounts along the entire length of the neuron (KOELLE 1955, GIACOBINI and HOLMSTEDT 1958, GIACOBINI 19 9, KOENIG and KOELLE 1961, FREDRICSSON and SJOQVIST 1962).

The sweat secretory fibres possess all these characteristics Their synapses were roughly localized to the stellate ganglion (LANGLEY 1891) many years before their cholinergic function was proved (DALE and FELDBERG 1934) Several authors have described the accumulation of eccrine sweat glands on the pads of the paws and the lack of such glands elsewhere in the cat (see SCHIFFFERDECKER 1922)

HELLMANN (1952) using the original KOELLE method (1950) failed to show AcChE in the sweat glands of the cat The cholinesterase demonstrated by him was classified as a non specific one As was pointed out subsequently by HURLEY SHELLY and KOELLE (1953) who demonstrated high AcChE activities around human eccrine sweat glands Hellmann s failure to discern AcChE was methodological in origin The present study has shown definitely that the enzyme around the eccrine sweat glands of the cat is AcChE Since the activity decreases markedly after denervation (see also HELLMANN 1952) it is probable that most of AcChE is located in the nerve terminals

The distribution of type I cells and the projection of their axons explain why LANGLEY (1891) obtained sweat secretion predominantly when the cranial end of the stellate ganglion was stimulated after the injection of nicotine It was known since LUCHSINGER s experiments (1878) that the sweat secretory fibres to the fore paw emerged through the brachial plexus LANGLEY in addition reported (1894) that secretory fibres probably can be present also in the 2nd grey ramus (cf Fig 5)

The present experiments show the correlation between the outflow of type I axons in the vertebral nerve and 1st thoracic grey ramus and the sweat secretory responses on the paw evoked by stimulation of these two nerves Such a direct correspondence is a primary condition for the assigning of any sweat secretory function to the type I neurons

The only non somatic cholinergic nerves known to exist in the fore leg besides the sweat secretory fibres are the vasodilator fibres (ELIASSON et al 1951) Studies of the cholinergic vasodilator pathway in the central nervous system have been carried out (see UVNAS 1961) but knowledge concerning their terminal synapses whether located in the paravertebral ganglia or peripherally is lacking Cholinergic fibres have also been demonstrated in the heart (FOLKOW et al 1948) but the localization of their cell bodies is unknown The present data do not exclude that the type I cells may give rise also to cholinergic vasodilator fibres This seems however less likely since big parts of the stellate ganglion totally lack such cells In this respect attention should be drawn to the type II cells which exhibit appreciable but lower AcChE activity than the type I cells These cells seem to be located in most sympathetic chain ganglia but they never constitute more than a few per cent of the cell population (FREDRICSSON and SJOQVIST 1962 SJOQVIST 1962)

The photomicrographs in Fig 2—4 show that the stellate ganglion is a compound ganglion consisting of a thoracic and a cervical portion (cardiac

zone) It is interesting to note that ECCLES (1943) by an electrophysiological approach has arrived at the conclusion that the cell bodies of the postganglionic fibres in the cardiac nerves are located within a small ventromedial part of the ganglion corresponding to the cardiac zone described in the present paper

The exclusive occurrence of type I-cells in the fore limb zones of the stellate ganglion and the correlation between their frequency and the outflow of sweat secretory fibres to the fore paw indicate that the type I cells and the sweat secretory neurons are identical Additional experiments are needed to prove this hypothesis and to decide whether the type I-cells may be related also to some other cholinergic function

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Pharmacological Analysis of Acetylcholinesterase rich Ganglion Cells in the Lumbo sacral Sympathetic System of the Cat

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Abstract

SJOQVIST F *Pharmacological analysis of acetylcholinesterase rich ganglion cells in the lumbo sacral sympathetic system of the cat* Acta physiol scand 1963 57 352—362 — Recent studies have indicated that AcChE-rich or type I cells in the stellate ganglion of the cat give rise to cholinergic sweat secretory fibres rather than vasodilator fibres The purpose of the present investigation was to prove this hypothesis Type I neurons were found in significant amounts exclusively in those lumbo-sacral ganglia that innervate the eccrine sweat glands of the hind paw The 7th lumbar ganglion always contained the highest proportion (10 8) whereas the other ganglia possessed lower amounts (S1 7 7 L6 3 5 and S2 1 9 Occasional type I cells were demonstrated in L5 but practically none in other lumbo-sacral ganglia The sweat secretory responses on the hind paw were always proportional to the number of type I cells in the stimulated ganglia The vasodilator responses evoked from ganglia crowded with type I cells and from those lacking such cells were on the other hand approximately equivalent The results form the basis for the conclusion that the type I-cells in the stellate ganglion and in the ganglia between L6 and S2 are identical with cholinergic sweat secretory neurons

It is known from earlier experiments that the fore limb zone of the stellate ganglion contains neurons with outstandingly high acetylcholinesterase activity (AcChE) i e type I-cells (SJOQVIST 1962 b) Evidence has been presented that such ganglion cells give rise to cholinergic fibres that are concerned with sweat secretion rather than with vasodilatation

The aim of the present investigation has been to exclude the latter possibility and more firmly link the type I cells to a peripheral sweat secretory function. Preliminary experiments have shown that the frequency of type I cells varies considerably in the different sympathetic ganglia between L5 and S2 (SJÖQVIST 1962 a). This fact offers an opportunity to compare sweat secretory and vasodilator responses in the hind limb as evoked from ganglia of different cellular composition.

Material and Methods

A. Anatomy and dissections

For nomenclature and variations in the size and location of the lumbo-sacral sympathetic ganglia see HOLMSTEDT, LUNDGREN and SJÖQVIST (1962). For histochemical purposes the ganglia were dissected out from the living cat, anaesthetized with sodium pentobarbital (NEMBUTAL, ABBOTT, 40 mg/kg i. p.).

B. Histochemical staining of AChE

After dissection the ganglia were immediately frozen in isopentane chilled with liquid nitrogen, kept at −70° C for 2—24 hours and sectioned in a cryostat (DITTE-HEMBERG) as described elsewhere (FREDRICSSON and SJÖQVIST 1962). Sections 7 and 10 μ thick were stained for AcChE by one of two alternatives depending on the aim of the experiment. For quantitative counting of type I-cells (see below) the ganglia were stained according to HOLMSTEDT (1957) with the appropriate controls and an incubation period of 30 minutes. To facilitate the counting of type II cells (see below) certain sections were counterstained with ammonium sulphide (KOELLE 1951) and incubation periods of both 30 and 60 min were used. By using serial sections corresponding parts of the ganglion could be studied after both types of staining and incubation periods.

C. Quantitative counting of different sympathetic cell types

Sympathetic ganglion cells of the cat are heterogeneous with respect to their relative AcChE activities when stained by the thiocholine method. A small proportion of the cells show heavy staining intensity (type I cells). They are specifically AcChE-rich as shown biochemically (HOLMSTEDT et al. 1962). Another small group of neurons exhibits a moderate degree of staining (type II-cells), whereas the great majority of ganglion cells display insignificant or no staining at all [illegible] SJÖQVIST 1962).

The cell counts were generally performed on serially sectioned ganglia [illegible] cellular composition of a ganglion may vary considerably [illegible] all ganglion cells in the section were counted, a total of about 2000 cells per [illegible] and was followed by the number of type I and II cells. The frequencies of the two cell types were expressed as percentages of the total [illegible] the same percentage of type I-cells [illegible] of such cells of the [illegible] magnitude of differences [illegible] consequently [illegible] lumbo-sacral ganglion with the exception of S2 [illegible] 0.97 1.00 0.93 0.30

The total number of experiments [illegible]

Table I Frequency of type I and II cells in different lumbo sacral sympathetic ganglia of the ca

Ganglion	Total number of counted ganglion cells × 10⁻³	Type I cells in per cent	Number of cats possessing type I cells/Total number of cats investigated	Type II cells in per cent
L 1	19 8	0 0? (11)	4/17	? 32 ± 0.87 (6)
L 2	15 2	0 05 (9)	3/14	1 68 ± 1 ?3 (6)
L 3	16 0	0 06 (9)	2/14	2 ?4 ± 0 68 (5)
L 4	21 1	0 0 (10)	3/15	2 85 ± 1.21 (6)
L 5	22 8	0 33 ± 0 37 (13)	9/21	2 27 ± 1 ?0 (9)
L 6	2? 2	3 50 ± 1 73 (15)	25/26	1 84 ± 1 03 (11)
L 7	28 9	10 80 ± ? 1 (15)	27/27	2 07 ± 0 42 (10)
S 1	14 5	7 ?0 ± 1 24 (15)	25/25	2 19 ± 1.23 (11)
S 2	10 6	1 90 ± 1 26 (9)	13/17	1 07 ± 0 66 (7)
S 3	5 9	0 04 (5)	1/ 9	1 ?6 ± 1.27 (5)

The values represent means ± S D per cat o means. Number of cats within parenthesis.
$\left(S\ D = \sqrt{\frac{\Sigma(\bar{x} - x)}{n}} \text{ when } n > 3 \text{ otherwise the denominator is } n-1 \right)$

qualitatively as well as quantitatively

D Studies of sweat secretion in the hind paw

The experiments were performed on 6 adult cats anesthetized with sodium pentobarbital (NEMBUTAL ABBOTT 20—40 mg/kg i p) Sweat secretion was produced by stimulating the various lumbo-sacral ganglia after they had been dissected free and isolated together with their pre and postganglionic fibres (see below) Supramaximal stimulation was obtained with a Grass stimulator model S4 and shielded silver electrodes The stimuli were applied during a period of 1—? min with a frequency of ?—15/sec and a duration of ?msec

The secretory responses were studied by the method of RANDALL (1946) The pads were painted with a 3 iodine solution and allowed to dry A starch paper was pressed lightly against the paw during the last 15 sec of the stimulation period The resulting blacking of the starch paper shows the proportional surface areas involved and also the degree of sweating Thus the size of the individual spots is an indication of the amount of secretion from each active sweat pore (see RANDALL 1946 Afterwards the stimulated ganglia were weighed and investigated histochemically for type I cells (codified preparations)

E Studies of choline gic vasodilatation in the hind limb

The experiments on the vasodilatation in the hind limb were performed on 15 cats weighing between 1 8 and 3 6 kg that were anesthetized initially with ether and followed by chloralose (30—?0 mg kg) in combination with 0 ur thane (0 3—0 5 g kg) The trachea was cannulated and the frequency of respiration recorded with a pressure tranducer (model PT5 Grass Instrument Corp Quincy Mass U S A) The arterial pressure and heart rate were recorded in the left or right femoral artery

recorded with an electronic ordinate writer (GOLDSCHMIDT and LINDGREN 1962) for graphic registration of blood flow The blood reentered the hind leg through the same artery cannulated about 2 cm more distally Clotting was prevented with heparin (25 mg/kg i v) The extracorporeal circulation was kept at about 37 C with a remote thermostat (Ultrathermostat Type U 3 Lauda/Tauber West Germany)

In the external iliac artery the muscle blood flow to the innervation area of the ganglia L5—S2 was measured Furthermore as calculated from textbooks of anatomy there seem to be no fundamental differences between the muscle volumes innervated by the ganglia L5—S1 which have been used for comparative studies of cholinergic vasodilator effects

The ipsilateral lumbo-sacral sympathetic chain was dissected carefully free through an anterior approach The trunk was ligated and cut between the 4th and 5th lumbar ganglia (L5) Through an electrode placed above L5 all preganglionic fibres to the ganglia below were stimulated since white rami are generally lacking below the fourth lumbar nerve (LANGLEY 1892 see also HOLMSTEDT et al 1962) Depending on the aim of the experiment either the sympathetic chain was left intact below L5 or the different ganglia between L5 and S2 were isolated by ligating and cutting the sympathetic chain immediately below each ganglion Thus 5 or 4 preparations were obtained (S2 was used only for sweat secretory experiments) each consisting of a ganglion with its preganglionic fibres and its grey postganglionic ramus (or rami) below

The vasodilator responses were studied predominantly in cats pretreated with reserpine (SERPASIL, CIBA) in a dose of 3—5 mg/kg administered i p 12—24 hours before the experiment (see ROSELL and ROSÉN 1961) To ensure maximal and constant vasodilator effects throughout the experiment guanethidine (ISMELIN CIBA 0 1—0 2 mg) was injected in the leg through an inlet in the arterial cannula In this way persisting vasoconstrictor effects were blocked Similar experiments were performed on cats treated exclusively with reserpine The cholinergic nature of the vasodilator response was evinced by its abolition after atropine (atropine sulphate 0 5 mg/kg i v) given at the end of some experiments

Supramaximal stimuli were applied for a period of 10—20 sec every 2nd minute with the following parameters duration 5 msec frequency 10—15/sec intensity 4—4 V

The pharmacological analysis of the localization of the peripheral vasodilator synapses was made in two ways In two cats treated exclusively with guanethidine (0 —0 3 mg i a in the limb) the intact sympathetic chain (from L5—S2) was painted with a 1 solution of nicotine bitartrate in 0 9 NaCl of 37 C Recording of the tail during stimulation gave evidence that the nicotine did not interfere with conduction in the fibres (see LANGLEY 1891) In the case of reserpinized animals (3 cats the different isolated ganglionic preparations were painted with nicotine and the electrode moved from the preganglionic to the postganglionic fibres in order to determine the site of the block. The effect of hexamethonium on the vasodilator responses was studied in three cats. Hexamethonium bromide (Vegolysen May & Baker Ltd Dagenham England) was injected i a in the limb in repeated doses corresponding to 0 1—0 2 mg 100 g muscle

Results

I Histochemical appearance of lumbo-sacral sympathetic ganglia

Data in Table I and the photomicrographs in Fig 1 show that the different lumbo-sacral sympathetic ganglia exhibit highly varying amounts of type I cells and that these cells are generally confined to the 6th and 7th lumbar the

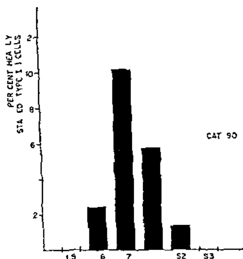


Fig 2 Typical frequency diagram of type I-cells in the lumbo-sacral ganglia L5—S3 (The relation between the absolut numbers of type I-cells in L6—S2 was 23 100 53 4)

1st and 2nd sacral ganglia The 7th lumbar ganglion invariably contains the highest frequency but in other respects the appearance of the frequency histograms can vary with different cats (Figs 2 and 4) As a rule the 1st sacral ganglion has considerably more type I cells than the 6th lumbar while the 2nd sacral ganglion shows the lowest frequency with occasionally no type I cells at all In rare cases only single type I cells are found in the 6th lumbar ganglion and in the cranial half of the 7th lumbar whereas the caudal part of this ganglion reveals a high density of type I cells (30 % or more) Generally the heavily stained neurons are located within a narrow region of the chain including the sympathetic trunk itself where the cells may appear abruptly in the caudal part of L6 and vanish just as suddenly in S2 Occasional cells may be found in the 5th lumbar ganglion of some cats and in exceptional cases single type I cells have been traced in other lumbo-sacral ganglia too In most instances however they are impossible to demonstrate above L5 and below S2 in spite of serial sectioning of the ganglia

As is apparent from Table I moderately stained type II cells are found in all ganglia in about the same frequency These cells are best recognized with prolonged incubation (Fig 1 L5)

II Sweat secretion in the hind paw after stimulation of different lumbo-sacral ganglia

An illustrative experiment is shown in Fig 3 and the corresponding frequency diagram in Fig 2 Stimulation of the isolated L5 was always without visible effect The sweat secretory responses evoked from the ganglia L6 L7 S1 and S2 were entirely dependent on the presence and the number of type I-cells in the stimulated ganglia The surface area showing the highest secretory response shifted from the inner to the outer part of the foot as the electrode was



Fig 3 Sweat secretory responses (Randall method) in the hind paw of cat 90 after stimulation of different lumbo-sacral ganglia The blacking of the starch paper shows the surface areas involved and also indicates the degree of sweating Note that the responses are dependent on the number of type I-cells in the stimulated ganglia (cf Fig 2) The comparatively weak response from S2 is due to its extremely small size

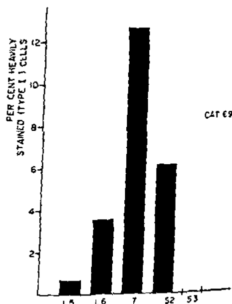


Fig 4 Another frequency diagram of type I-cells. In this case the 1st and 2nd sacral ganglia are fused Characteristic peak in L7

moved from higher to lower spinal levels although an overlapping existed between the different ganglia Stimulation of L7 always resulted in a copious secretion and mostly over the whole foot The secretory responses evoked from S1 were generally appreciably greater than those produced from L6 The responses from S2 were less pronounced and weaker than might be expected on the basis of the relative frequency diagram This is due to the very small size of this ganglion Consequently its absolute number of type I-cells is especially low

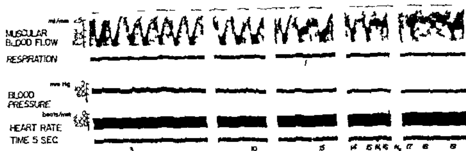


Fig 5 Cat 69 Vasodilat esponses in a hind limb to supramaximal stimul t on of different lumbo- cral symp thetic gangl a W ght 2 8 kg Anesthes a chlo al se 30 mg kg s ppl m nt ed th 20 u than 0 2 g/kg v Reserpin 5 mg kg i. p 12 hours befo the experiment. Th asodilat component unmasked after blocking of persisti g vasoconstricto ffects w th guan th din 0 2 mg a. Muscular blood fl w r co d d in th right external ilia art ry blood p essur in left f mo al artery
1—7 Sympatheti chain l gat d and cut below L4
1—? Stimulation abo e L5 asodilatat on produ ed from the ganglia between L5 and S2
3—5 Stimul t n bove L7 e asodil tation p od ed from L7 and S1—S2
6—7 Stim l t n bo L5
A Sympath t chain l gated and cut betw en L6 and L7
8—10 St mulation abo L5 Th d uble peak f type I-cells in L7 and S1—S2 limin ted asodilatati ns persist
B The diff t ganglia isolated.
11 and 13 Stimul ti n of isol t d L6
12 Stimulat n f sol ted L5
14—18 P egangl m timulation of solated L6
N and N Carefl l painting of L6 th mall amounts of 1 n tin
17—18 No asodilatat ons w th pregangli nic timulat n of L6
19 El ct od shifted to th postgangli nic ramus Rest ti n of asodil tor esponses
N t that th asodilat esponses from th different ganglia are of the same o d r of magnitude and ind pendent f th fr q ency of type I-cells in th timulated gangl a (see Fig 4)

III Pharmacological analysis of the localization of the vasodilator synapses

The vasodilator responses in the hind limb to preganglionic sympathetic stimulation could be blocked by applying nicotine to the sympathetic chain (Fig 5) Depending on the type of experiment the ganglionic site of the block was evidenced either by the restoration of the vasodilator effects with post ganglionic stimulation (isolated ganglia reserpinized cats) or by the persistence of piloerection of the tail during the stimulations (intact sympathetic chain guanethidine i a in the limb) Furthermore intraarterial doses of hexamethoni um (*equivalent to 1—2 mg/kg of muscle*) did not diminish vasodilator responses evoked immediately after the injection

IV Cholinergic vasodilatation in the hind limb produced from ganglia with varying cellular composition

A representative experiment is seen in Fig 5 This and the subsequent six experiments showed that when the chain was divided above L7 and conse quently the majority of type I cells excluded from stimulation (see Fig 4) the resulting decrease in the vasodilator response was astonishingly small In fact

the combined vasodilatation evoked from L5 and L6 was approximately equal to that produced from the combined stimulation of L7 and S1—S2 No quantitative differences were found between the vasodilator responses produced from the isolated ganglia L5 L6 L7 and S1 in spite of their highly varying amounts of type I cells In other experiments the vasodilator effects produced from an isolated L5 lacking type I cells were equal to those evoked from a ganglion exhibiting a high density of type I cells such as L7

Discussion

A striking correlation has previously been demonstrated between the occurrence and localization of AcChE rich or type I cells in the stellate ganglion and the outflow of cholinergic sweat secretory fibres to the fore paw of the cat (Sjöqvist 1962 b) It could not be determined with certainty however whether the type I cells were exclusively concerned with sweat secretion or were related possibly also with vasodilatation

The suggestion has recently been made that the peripheral cholinergic vasodilator synapses may be located in the skeletal muscle vessels (Szücs-Hetényi and Went 1957) This postulate is based on highly indirect evidence and seems hardly tenable from a pharmacological point of view The present experiments show that the vasodilator responses to electrical stimulation can be blocked by an application of nicotine to the ganglia and evoked again by postganglionic stimulation Furthermore intraarterial injection of hexamethonium into the hind limb does not cause a reduction in the vasodilator responses These data indicate that the final cholinergic vasodilator synapses are located within the sympathetic chain

Although located in the chain ganglia the postganglionic vasodilator neurons seem to be completely unrelated to the type I cells This is shown by comparative studies of vasodilator effects produced from different isolated ganglia Postganglionic vasodilator fibres seem to emerge from each of the different ganglia in about equal numbers and in any case totally independent of the occurrence of type I cells Consequently the vasodilator fibres (see Uvnäs 1961) the only known cholinergic autonomic structures in the limbs besides the sweat secretory fibres (Dale and Feldberg 1934) probably do not arise from scattered type I cells in the sympathetic chain In this connection the finding that moderately stained type II cells occur in about equal numbers (about 2 %) in most paravertebral ganglia is of interest (Sjöqvist 1962 c)

The functional significance of the present results becomes evident also when we examine closely a 70-year old paper by Langley (1891) After having localized the sweat secretory neurons supplying the hind paw to the sympathetic ganglia between L6 and S2 Langley states as a rule the 7th lumbar grey ramus contains rather more secretory fibres than the 1st sacral grey ramus the grey ramus of the 6th lumbar nerve contains comparatively few secretory

fibres but rather more than the grey ramus of the 2nd sacral LANGLEY's rough mapping of the secretory neurons was based on qualitative observations of sweat secretion Even the exceptions to the rule quoted above can however be explained by the occurrence and frequency of the type I cells These exceptions pertain specifically to the variations in the outflow taken by the secretory fibres from L6 and S2, and to those cases in which Langley found that a few secretory fibres join nerve cells in the 5th lumbar ganglion pass down the sympathetic and issue by the grey ramus of the 6th ganglion This *latter arrangement explains why stimulation of the isolated L5 (i e when* containing type I cells) never produces any secretion in the paw On the basis of Langley's observations it is probable that the axons of those single type I cells which are found in ganglia of a few cats above L5 and below S2 pass through the sympathetic trunk and are carried peripherally by the grey rami arising from the sweat secretory ganglia

The present experiments demonstrate the absolute correspondence between the sweat secretory responses in the hind paw and the frequency of type I cells in the stimulated ganglia It is apparent that the unique distribution of AcChE rich neurons in the lumbo sacral ganglia L6—S2 and in the sweat secretory regions of the stellate ganglion (SJÖQVIST 1962 b) entirely parallel the outflow of cholinergic fibres to the eccrine sweat glands As other cholinergic neurons the type I cells in the lumbo sacral region are characterized by outstandingly high AcChE activity as shown histochemically and biochemically (HOLMSTEDT *et al* 1962) The calculated mean difference in AcChE activity between these cells and adrenergic sympathetic neurons is estimated to be about 30 fold (SJÖQVIST 1962 c)

This investigation is probably the first one in which a particular type of sympathetic neuron has been linked to a special peripheral function Earlier attempts to divide sympathetic ganglion cells into functional groups have been unsuccessful because of the cytoarchitectonic uniformity of all neurons (see HILLARP 1960) including the AcChE containing ones (FREDRICSSON and SJÖQVIST 1962) It would be of value to map out the whole sympathetic system histochemically with the thiocholine method Such a study is under way and the available data show that no other regions than those innervating the paws contain type I cells in considerable amounts (SJÖQVIST 1962 c) This can be explained by *the unique restriction and accumulation of cholinergic eccrine* sweat glands on the paws of the cat (SCHIEFFERDECKER 1922)

In summary the author's findings tally well with data from studies by LANGLEY (1891) DALE and FELDBERG (1934) They form the basis for the conclusion that the type I cells described constitute the cell bodies of cholinergic sweat secretory neurons the classical exception in the cat's sympathetic system as regards neurohumoral transmission

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The Participation of Bone in the Sodium and Potassium Metabolism of the Rat I

Simultaneous determination of the exchangeable body sodium and potassium and the exchangeable and inexchangeable fractions of these ions in bone in the normal rat

By

N NORMAN

R c ed 23 August 1962

Abstract

NORMAN N *The Partic p n f b ne i the d um and potass m met b lism f the at I* Acta ph s ol. scand 1963 57 363—372 — A method s described wh reby total ex hang abl sod um and potas s um in the at s s multaneously d t rmin d by the use of isotope d lut on techn ques These m asurem nt ha been comb ned with the determ nation of th total and ex hang able concentrat ons of sod um and potass um in bon made poss bl by a new p oc dure employing cat on exchange resin fo isolation of the bone lect olytes Ser es of ormal 100 g rats 200 g at and 300 g ats re examined Th results fo the total body ontent of sodium and potass um obtained n these an mals agr ed w th published data from total body ashing p o edures Total bone odium con ent at on ncreased w th age w h as th conc nt ation of the ex hangeabl fraction was rather constant n the thr g oup Th exchang ab l ty of the bone sodium was 71 per ent n th 100 g ats and 56 and 50 pe c nt n the 200 and 300 g ats Bone potass um con entration was highest n th 100 g at th exchang ab l ty of this on in bone was 80 per cent n this group and clos to 100 per ent in the t o other groups The complete set of figu es is g ven in Tabl III

Present add ess Al Hos ta) O lo N rw y

Since the early studies of the distribution of sodium and potassium in the living organism using the radioactive isotopes of these ions (JOSEPH COHN and GREENBERG 1939 GREENBERG CAMPBELL and MURAYAMA 1940) a number of contributions in this field far too great for quotation here have been published (for general orientation the reader is referred to the review given by MOORE 1954) To our knowledge no study of simultaneous determination of the total exchangeable body sodium and potassium has however, been performed in as small an animal as the rat nor are we aware of any publication in which these measurements have been combined with studies of the total and exchangeable concentration of sodium and potassium in bone In this paper a method for such a combined simultaneous determination is described and results obtained in normal rats are presented In other experiments the same method was used to study variations in electrolyte intake acidosis and alkalosis adrenalectomy and various hormonal treatments (NORMAN 1963 a b)

Material and Methods

Hooded male rats of the Royal Victoria Hospital strain were used For the metabolic experiments they were of the 200 g size (range 198—202 g) but two groups of untreated 100 and 300 g rats respectively were included in order to provide information on the influence of body size and age

For technical reasons only 8 rats could be studied at a time In order to avoid statistical bias this group was always a composite of 4 different types of metabolic experiments, 2 rats from each series

The standard diet in the rat colony was Purina Fox Chow (a commercial product containing 0 15 meq/g (0 35 per cent by weight) of sodium and 0 20 meq g potassium (0 8 per cent by weight)) given *ad libitum*

During the experimental period the rats were kept in a room provided with constant temperature humidity and light conditions throughout the year The breeding station in the same building was air conditioned

Flame photometry

The sodium and potassium determinations in the various fluid samples from plasma, bone and urine were performed on a Baird Associates Flame Photometer with internal lithium standard By frequent use of the standards within the actual ranges of the measurement it was possible to limit the error of the readings to between 0 1 and 0 133 per cent for sodium and to between [illegible] and 0 4 per cent for potassium. The poorest accuracy was associated with the lowest concentrations

Radioactivity determinations

For the measurement of the radioactivity in the plasma and bone fractions after the ion exchange chromatography which left the specimens to be tested in solution a 20th Century Electronics well counter tube (Type M4) was employed This tube as well as the Geiger tube used for the urine (see below) was connected to an [illegible] Glow Tube Scaler with super stable high voltage supply and automatic timer ([illegible] Instrument Company Cambridge 39 Mass. U S A) a preamplifier model [illegible] being interposed in the circuit Because of the great number of determinations on the days when the rats were examined and the generally heavy program on these days, it

was not possible to prolong the counting times of the fluid sampl s to such an extent as to obtain the highest degree of accuracy For the measurements in the flu ds containing Na the error varied from ± 2 per cent to ± 3 per cent for K from ± 2 5 per cent to ± 5 per c nt the poorest accuracy be ng associated ith the lowest concentration

Th amount of radioacti ity n the urine was determined n dried samples on aluminum planchets by the use of an end w ndow Ge ger tube (Type D33 Nu l r Instruments and Chemical Corp Chicago) The method for obtain ng the individual counts from the ²Na and K by using a th n aluminum filter is described by ARONS and SOLOMON (1954) The most practical way of tackl ng such problems as elf absorption back scatter ng f om the sample mount and source scattering is to use ference tandards that in fo m and concentration esemble the test amples This as done throughout and the counting rate was kept within the optimal range of the instrument

Total b dy xchan ble sod m and potass um

Th s quant ty oft n called the total xch nge ble space [1] ol th electrolyte is det rmined and defined for each of the lectolytes by the equation

$$T = (I - I) / S$$

(T — Total body exch ele trolyte I = Injected otope (μC) I = Urine isotope (μC) S = Sp cific activity (μC/meq))

The quat on s applied at the end of tl e equil brat on per od of the radio otope in the organ sm The basis fo ts val dity is the as umpt on that at th s time the rad o sotope s evenly d t buted (among th atoms of the co espond ng el ment) n the body The specif c act v ty (rat o rad oa t e/non radioact e element) is obt n d from a sample of the total pa pla ma has b en most frequently used The u ne isotope is th part of the total injected radioact ty lost n the u ne du ng the equ l brat on period No ad oact vity from Na was ever d sco ered n the faeces The K in the faeces vas n ver above 1 per cent of the inje ted amount and usually ell below 0 5 per c nt The examinat on of the faeces was the efore d scontin ed aft r a easonable p od of trial

The two sotopes ver given separately by intraperitoneal njections Eacl t received bout 10 μC Na n 1 ml sotonic odium chlorid s lut on and ab ut 20 μC ²K in 2 ml isoton potas um chlor d solution Syr ng s equipped with special adaptors llo ed th admin tr t on of produc ble and dentical volumes The same volumes we e measured nto s pa ate olum t c flasks which served as ef ence standards fo tl e part cula exper ment

Th urine was collc t d in metabol c cages dur ng the 15 hour equilib ation period The net and funn l of the cages e washed w th distill d water and w th the a d of sodium analys s of the washing wat r lo ses du to the urin drying on th se parts could be calcul ted

The r ts re bled to death by ao t c punctur w th a n dl and syringe bar ly mos t ned w th hepar n and under th anesthes a The blood was mm diately centr fuged In 1 ml plasma th p ot n was pr p t t d w th 1 ml of 10 per cent of tricl lo cetic acid Th supe natant aft centrifug t on tog th r w th a fu th r 1 ml of tr chlo cet c ac d used fo wash g of th p ecip tat was added to a column of cat on exchange es n (Dowex 50 minus 400 mesh 12 per nt cross linked) pr v ously washed with hydrochl c a d and dist lled ater The resin in a column 6 cm h gh and 1 cm in diamete was found to be ad quate fo the separat on of the sod um and potass um ions Elut on was performed th 0 25 N hyd ochloric acid at a rate of 0 75 ml per m nut

[1] Th term p ce has g n d ceptance n this fi ld of in est g t n in the meaning of amount d n t ferr ng t lum As can be f m the formul t is h p essed in rms f m q

Table I Recovery of sodium and potassium following separation in a cation exchange column from 6 samples of plasma and in 6 tests with an artificial salt mixture with a composition identical to that of bone

	Befo e column Mean ± S D	After column Mean ± S D
Plasma		
Sodium (mEq/l)	147 44 ± 5 22	144.26 ± 4 42
Potas um (mEq/l)	3 86 ± 0 02	3 89 ± 0.24
Bone		
Sodium (mEq)	0 05	0 0 05 ± 0 0007
Potass um (mEq)	0 01	0 0094 ± 0 00007

All the sodium was found in the fraction from 35 to 125 ml in the eluate and potass um could afterwards be washed out rapidly with 1 N hydrochloric acid The whole separation required 3 ¹/ hours

The recovery in a series of 6 samples of rat plasma determ ned by flame photometry before and after having passed the columns is shown in Table I The fractions were subjected to radioactivity measurement as well as flame photometry and the pec fic activity (ratio radioactive/non radioactive element) calculated

In addition to this determination in plasma a further 1 ml of plasma was al ays subjected to flame photometry and served as a check on the results obtained fter il separation of ionic exchange

The bone procedu e

A detailed description of the bone procedure has been publish d (NORMAN LLCF and BROWNE 1957) The femoral diaphyses from which periosteum and bone marrow had been removed were ighed immediately dissolved in concentrated nitr c aci l dil ted with ater and appl ed to a column of the same resin as used for separation of sodium and potassium in plasma

The full recovery from ar artificial salt mixture of a compos tion sin lar to that of bone appears in Table I

Results

Experiments for further evaluation of the method

In order to establish the adequate equilibration time for the isotopes in the rat body and to test the reproducibility of the determination of the total exchangeable sodium and potassium the latter were determined twice in the same rat with a time interval The first blood sample was obtained by heart puncture and the second when the animal was killed The sodium paces were found to be identical in the same rat at various times between 10 and 18 l o the mean value in the 200 g rat was close to 9 meq The potassium spaces were not reproducible to the same extent The results in a series of 11 rats measured at 15 and 20 hours after the injection of isotopes were 12 11 ± 1 14 meq and

Table II Tissue specific activities (μC/mEq) in various tissues following different equilibration periods of ^{24}Na and ^{42}K. The urine values are from the total dose during the equilibration period

	Sodium Mean ± S.D	Potassium Mean ± S.D
100 g rats, 15 hours equilibration (8 animals)		
Plasma	1 9 92 ± 0 120	2 6//0 ± 0 360
Bone	1.3664 ± 0 071	2 1866 ± 0.258
Urine	0 6174 ± 0 107	1 2 8 ± 0.377
200 g rats, 15 hours equilibration (18 animals)		
Plasma	1 049 ± 0 055	2 7030 ± 0 327
Bone	0 5950 ± 0 040	2 007 ± 0 435
Urine	0 842 ± 0 1 9	3.5266 ± 0.371
300 g rats, 15 hours equilibration (8 animals)		
Plasma	0 876 ± 0 058	1 9214 ± 0 180
Bone	0 38 8 ± 0 038	1 6533 ± 0.270
Urine	0 6058 ± 0 094	2 9001 ± 0.256
200 g rats, 15 hours equilibration (6 animals)		
Plasma	1 096 ± 0 049	2 9659 ± 0 0110
Muscle	1 089 ± 0 044	2 9088 ± 0.408
Brain	1 116 ± 0.097	1.2295 ± 0.213
200 g rats, 36 hours equilibration (8 animals)		
Plasma	1.2539 ± 0.267	—
Bone	0 7 06 ± 0 136	—
200 g rats, 15 hours equilibration (6 animals)		
Plasma	—	2 9601 ± 0 401
Red cells	—	2 1139 ± 0 121
200 g rats 0 hours equilibration (6 animals)		
Plasma	—	3 1082 ± 0.355
Red cells	—	2 3052 ± 0 171
200 g rats 15 hours equilibration (6 animals)		
Plasma	—	?50 9 ± 0 1 2
Liver	—	2.5192 ± 0 171
Stomach wall (pylorus)	—	2 7735 ± 0 372
Kidney	—	2 602. ± 0 183

11 43 ± 0 48 meq respectively. The reasons for the variations will be discussed below.

Table II gives the results for specific activities in various tissues compared with those of plasma after different periods of equilibration.

It appears that Na exchanged completely in muscle and brain. In bone the specific activity was lower than in plasma; the ratio bone specific activity to plasma specific activity was 0 71, 0 57 and 0 49 in 100 g, 200 g and 300 g rats respectively. The figures for total and exchangeable bone sodium concentration

Table III Results obtained usin, the presented double isotope dilution technique in three groups of rats selected by weight

	Number in the gro p 8	18	8
	Mean ± S D	Mean ± S. D	Mean ± S D
Orig nal we ght of rats (g)	100 ± 2	200 ± 2	300 ± 3
Final weight (g)	93 8 ± 4 8	1 6 1 ± 8 0	277 8 ± 2.9
Total exch. body Na (mEq)	4 /4 ± 0 32	9 03 ± 0 72	12,06 ± 0.51
Total exch. body K (mEq)	6.52 ± 0 73	13 28 ± 1.2	19,58 ± 1 9
Urine olum (m l)	7 1 ± 2 6	26 0 ± 26 0	10 9 ± 5 6
Urine Na (mEq)	1 51 ± 0 46	0.81 ± 0 3	0 93 ± 0.3
Urine K (mEq)	1 37 ± 0 2	0.90 ± 0 ?5	0 97 ± 0.23
Total bone Na conc. (mEq kg)	143 4 ± 4.2	203 6 ± 13 1	210 4 ± 8./
Exch. bone Na conc. (mEq/kg)	101 4 ± ? 8	115 0 ± 8 8	104.2 ± 8 0
Total bone K conc. (mEq/kg)	39 3 ± 7 5	6 0 ± 2 4	21 1 ± 2 0
Ex h. bon K conc. (mEq/kg)	31 0 ± 5 3	?5 7 ± .5	18.1 ± .7
Plasma Na conc. (mEq/l)	14 9 ± 1 5	148 0 ± 1 6	146 8 ± 1 9
Plasma K conc (mEq/l)	3 7 ± 0 19	3 8 ± 0 60	3 7 ± 0 43

Th rats recei ed only water during th last 15 hours in the metabol c cages, there as, therefore, a eight loss in this per od.

Urin volume collected in th qu l brati n per od during th 15 hours in the metabo c cages.

E p essed as mEq kg wet bone.

in Table III sho that the decrease in bone specific activity in the larger rats was a result of an increase in the inexchangeable fraction the absolute value of the exchangeable fraction was rather constant The ratio bet een bone and plasma specific activities after 15 and 36 hours equilibration did n t differ significantl The absence of an increase with prolongation of equilibration time agrees ith the findings of EDELMAN JAMES and MOORE (195?) and BAUER (1954 a)

Complete exchange with ^{42}K occurred in muscle liver stomach wall and kidneys after 15 hours but not in brain and red cells (Table II In the latter a significant increase (P 0 05) in specific activity took place with 40 hours equilibration CORSA *et al* (1951) demonstrated that 40 hours as required for complete exchange in the red cells The specific activity of bone potassium did not differ significantly from that of plasma in 200 and 300 g rats In 100 g rats there was a small inexchangeable fraction after 15 hours equilibration There as a remarkable difference bet een the urinary potassium val es in 100 g 200 g and 300 g rats The first group had a low specific activity in t e latter t o it was higher than in plasma

Complete data obtained from 100 g 200 g and 300 g rats

Calculated on the basis of the results in Table III the total exchangeable body potassium per kg was 69 5 meq 71 4 meq and 69 8 meq respectively in the three groups The same constancy was not found for sodium the values per kg rat were 50 5 meq 48 5 meq and 43 4 meq for the 100 g 200 g and 300 g groups The large standard deviation in the urine volume of the 200 g rats was due to a diuresis of more than 50 ml in 4 of the 18 animals The urinary loss of sodium and potassium during the equilibration period was highest in the 100 g group where the excretion was 4 times that in the 300 g group on a per kg weight basis Assuming the toal skeletal weight in a 200 g rat to be 22 g (SKELTON 1927) and using the figures for exchangeable and inexchangeable bone sodium concentration in such a rat it can be calculated that bone contains 1 938 meq of sodium that does not exchange and 2 541 meq that exchanges Total bone sodium amounts therefore to 4 479 meq as compared with a total body content of 10 963 meq (total exchangeable body sodium plus inexchangeable bone sodium) In consequence bone sodium amounts to 40 8 per cent of the total sodium in the 200 g rat The bone potassium concentration total as well as exchangeable decreases with age

Discussion

Some of the limitations of the method will be apparent from the considerations presented above For sodium it seems justifiable to conclude that the total exchangeable body measurement includes virtually all body sodium except the inexchangeable fraction in bone For potassium full equilibration was not reached in 15 hours in the red cells and the brain however the blood accounts for only 5 5 per cent and the brain for 0 46 per cent of the body weight of the rat (SKELTON 1927) It is justifiable to assume therefore that the dilution technique in the case of potassium measures more than 95 per cent of the total amount in the body The values obtained for the total exchangeable sodium and potassium agree well with those of CHEEK and WEST (1955 1956) CHEEK, WEST and GOLDEN (1957) and BERGSTROM (195) based on total body analysis In comparing the sodium figures the inexchangeable bone sodium that is included in their analysis must be taken into account It is interesting to note that larger standard deviations also occurred in their potassium than in their sodium values In Table III the standard deviation for the total exchangeable potassium increases with the size of the rat Similar increases in standard deviation do not occur in the sodium spaces and in the final weights indicating a greater variation in the total body potassium than in the total body sodium in rats of the same size It does not however explain the large variation in the repeated potassium space determination in the same rat at 15 and 20 hours equilibration Some of this variations was without doubt caused by the inaccuracies in the counting and flame photometry of the rather low concentra

tions of plasma potassium An increase in the potassium space at 20 hours would be accounted for by hemolysis and release of potassium of low specific activity at the time of the heart puncture at 15 hours *equilibration* A fall in the size of the space can only be explained by the introduction into the vascular compartment of potassium with higher specific activity Such added activity would be noticeable even if very small because plasma potassium is a negligible fraction of the total body potassium

The high urinary specific activity of potassium in 200 and 300 g rats (Table II) is probably due to the rapid excretion of the injected tracer and carrier The relatively much lower specific activity in the urine of 100 g rats may be explained as follows The 100 g rats are thin fast growing and when exposed to starvation in the metabolic cages they will quickly and heavily draw on the liver glycogen stores with resulting release and excretion of considerable amounts of non radioactive potassium (Table III shows the high urinary sodium and potassium of the group) The potassium storage with glycogen in the liver was well demonstrated by FENN (1939) The sodium excretory effect of a potassium load was shown as early as in 1873 by BUNGE

When the bone sodium and potassium values are compared with these presented in the literature they correspond very closely to the results obtained by BERGSTROM (1952 1954) and BERGSTROM and WALLACE (1954) Also in Bergstroms results the variation from rat to rat is very small in contrast to a number of publications in which very large differences are found from sample to sample The results are in agreement with those of BAUER (1954 b) MUNROE IATOSKAR and WILSON (1957) and NICHOLS and NICHOLS (1958) when the difference caused by the fact that their results are given as meq/kg dry bone is calculated

The water content of the bone samples from 200 g rats was found to be about 30 per cent The distribution of this water between extracellular fluid intracellular fluid and crystal water (hydration shell and hydronium ions on the bone crystals) is difficult to assess

HARRISON DARROW and YANNET (1936) originally determined the excess sodium of bone as the difference between total bone sodium and the sodium in the extracellular space the latter being determined by the chloride content of the sample Other studies have revealed that as much as 33 per cent of the tendon chloride is located outside the extracellular fluid of the tissue (LEVITT et al 1956) In view of the close resemblance in composition between tendon and organic bone matrix calculations based on the assumption that the chloride is entirely in the extracellular fluid are of doubtful value The magnitude of the sodium concentration in the bone matrix (organic and inorganic) can however be roughly estimated On the assumption that the intracellular fluid does not contain sodium the excess sodium in the 200 g rat can be calculated to be 189 meq/kg if 1/3 of the bone water is extracellular fluid In the case of 2/3 of the bone water being extracellular fluid the excess sodium will be 174

meq/kg The latter estimate corresponds to the value obtained by calculation from the chloride space A similar estimate for bone potassium can be made If 1/3 of the bone water is intracellular fluid with a potassium concentration of 150 meq/l approximately 11 meq/kg of the 26 meq/kg bone potassium is located in the bone matrix

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Th s art cle as ell as th f ll wing t o art cles in this series f rm part of a thesis submitted in partial f lfilm nt fo the d gree of Ph D M G ll Uni ers ty I ish to express my incere gratitude for the valuable ad ice and gu dan e of Professor J S L BROWNE and Dr J C BECK n whose depa tment the w k as carried out My best thanks are extended to the entire t ff of the Endocrinol g cal Labo atory Royal V ct ria H sp tal Montreal for the r fr endly co-oper ti To Mrs ANNE LI E GEHRLICH I am part cularly indebt d for her excellent work as a t chn l assistant.

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The Participation of Bone in the Sodium and Potassium Metabolism of the Rat II

The effect of variation of electrolyte intake acidosis and alkalosis

By

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Abstract

Norman N *The participation of bone in the sodium and potassium metabolism of the rat II* Acta physiol scand 1963 57 373—383 — Rats were examined according to a previously described technique (Norman 1963) permitting the simultaneous measurement of total exchangeable sodium and potassium in their body as well as the total and exchangeable concentration of sodium and potassium in their skeleton The effect of various combinations of sodium depletion potassium depletion sodium loading acidosis and alkalosis was studied Simple sodium depletion caused a loss of 10 per cent of body sodium 90 per cent of the loss was accounted for by shrinkage of the extracellular fluid volume When acidosis was induced in addition sodium was given off from the exchangeable bone sodium fraction Potassium depletion caused a picture similar to that of starvation The potassium depleted rats had a greatly reduced ability to handle a metabolic alkalosis The participation of bone sodium and potassium in the metabolism of the rats was smaller than found by others The mean difference from maximum storage of sodium in alkalosis to maximum depletion in acidosis corresponded to 10 per cent of the total bone sodium concentration Bone potassium concentration showed great constancy with no parallel to the variations in bone sodium

The interest in the participation of bone in sodium and potassium metabolism has mainly been focused on the potential of this structures a reservoir for the electrolytes from which they could be supplied in case of need and where

they could be stored in periods of surplus In most of the published studies the investigators have concentrated on the significance of bone as a source of extra sodium mainly during metabolic acidosis (BERGSTROM 1953 NICHOLS and NICHOLS 1953 1958 LEVITT and TURNER 1953) Considerable loss of sodium and potassium from the bone in electrolyte depletion without acidosis has also been found (BERGSTROM 1954 1956 NICHOLS and NICHOLS 1955 1956 EDELMAN JAMES and MOORE 1952 WOODBURY 1953 MUNROE SATOSKAR and WILSON 1957) Nichols and Nichols were able to demonstrate a gain in total bone sodium by chronic sodium loading Studies on the participation of bone in alkalosis and potassium depletion have to our knowledge not been published

Experimental Procedure

Methods

For methods and calculations the reader is referred to NORMAN (1963) Male rats of the hooded Royal Victoria Hospital strain weighing between 198 and 202 g were used throughout.

The diets

The standard diet in the rat colony consisted of Purina Fox Chow and water The same diet was fed to the normal control rats in the metabolic experiments. The sodium and potassium content in this diet was determined by flame photometry to be in the range of 0 15 meq/g and 0 20 meq/g respectively Expressed as per cent of the weight of the food sodium represented 0 35 per cent and potassium 0 8 per cent

The low sodium diet was composed of the following ingredients

Powered whole egg	10 per cent
Yeast	8 »
Casec (dried skim milk)	10 »
Canada approved bread flour (wheat)	32 »
Corn Starch	40 »

Water was added to the mixture and the dough was baked to a fairly hard crackerlike consistency Vitamins and minerals were not added to this diet as it was only given for short periods It was given to the rats *ad libitum* in the same way as Purina Fox Chow The animals ate approximately the same amount of this low salt diet as of the standard food The sodium and potassium content was determined in the ingredients as well as in the mixture The results were in close agreement the mixture contained 0 05 per cent sodium and 0 35 per cent potassium

The low potassium diet had the following composition

Corn starch	60 per cent
Canada approved bread flour	0 »
Mazola corn oil	15 »
Casec (dried skim milk)	5 »
Poly Vi Sol (Vit. A 3,000 I U ml Vit D 16 0 I U ml Ascorbic acid 60 mg ml Thiamine 1 7 mg ml Riboflavin 1 3 mg ml Niacinamide 10 mg ml)	0 1
CaHPO	2 »
NaCl	1 5

The mixture was prepared and presented to the rats in the same way as the low sodium diet They did not particularly like the low potassium diet ate very little of it and lost considerably in weight from the second and third day onwards The electrolytes were determined and the content was 0 06 per cent potassium and 0 6 per cent sodium.

Sodium loading was introduced by the administration of 0 45 per cent sodium chloride and 5 per cent glucose in the drinking fluid Normal rats would drink between 100 and 200 ml of this fluid in 24 hours 200 ml of the fluid contained 15 meq of sodium almost double the total exchangeable sodium in the rat The salt glucose drinking fluid was continued during the last 15 hours in the metabolic cages in the rats receiving the sodium loading procedure All other rats received only water in this period

Acidosis and alkalosis

Acidosis was induced by intraperitoneal dialysis against a solution containing 90 meq/l of ammonium chloride and 5 per cent glucose In the first experiments 20 ml were injected into rats receiving a normal diet equilibrated for 4 hours and then removed This was tolerated well but very small changes were observed in the bones of these rats When the same procedure was used in rats on the low sodium diet the mortality was very high In such rats therefore an equilibration period of 1 hour with 20 ml or 2 hours with 10 ml of the solution was used The same amount of sodium and potassium was recovered from the peritoneal cavity in the two instances These were (recoveries and standard deviation) for sodium $1\ 12 \pm 0\ 25$ meq and for potassium $0\ 052 \pm 0\ 015$ meq

Alkalosis was produced by the injection intraperitoneally of a solution containing 300 meq/l of sodium bicarbonate The rats which had been on the standard diet received 20 ml of the fluid the ones on low potassium diet 15 ml (i e 10 per cent of body weight in both instances) The solution was removed after 3 hours and the sodium potassium and chloride content determined. Urine was collected during the intraperitoneal dialysis

The radioactive tracers were injected immediately following the procedure and the the rats transferred to the metabolic cages

Results and Discussion

Low sodium diet (Table I Groups 2 and 3)

The treatment caused a statistically significant fall in the total exchangeable body sodium (Group 1 versus 2 P—0 01 and Group 1 versus 3 $0\ 05 > P > 0\ 01$) The fall in total exchangeable body sodium was associated with a small reduction in body weight In Group 2 there was also a slight fall in total and exchangeable bone sodium concentrations in contrast to the normal levels in Group 3 The changes resulting from the low sodium diet were more marked after 3 days than after 5 days indicating perhaps that the rat needed some time to adjust to the reduced salt intake The smaller loss of sodium in the urine in Group 3 may support this view The concentration of total and exchangeable potassium in the bone in Group 2 was significantly (P—0 01) below control levels the mean value for the total exchangeable body potassium of the group was also below that of the controls but this difference was not statistically significant

The rats ate well of the low sodium diet and thrived on it The results were therefore not those of partial starvation The difference from starvation was observed in a small group of rats given water only for 48 hours The weight of these rats fell to 175 g and they uniformly lost 1 5 meq of sodium and from 1 15 to 2 meq of potassium

A reasonable calculation of the extracellular fluid volume of the animals can be made on the basis of the total exchangeable sodium of the body the exchangeable sodium concentration of bone and the plasma sodium concentration The total amount of sodium in the extracellular space is the difference between the total body exchangeable sodium and the sum of intracellular sodium and exchangeable bone sodium This difference divided by the sodium ultrafiltrate concentration gives the extracellular volume According to CHEEK WEST and GOLDEN (1957) the serum ultrafiltrate concentration is obtained from the plasma concentration by the following formula

$$(Na)_f = \frac{(Na)_{pl}}{0.93} \times 0.95$$

$(Na)_f$ represents ultrafiltrate sodium concentration $(Na)_{pl}$ plasma concentration 0 93 is the introduced correction for plasma water and 0 95 the correction for the Donnan equilibrium The amount of extracellular sodium is small it occurs in low concentrations in muscle (COTLOVE *et al* 1951) and red cells and not at all in the cells of the viscera (CHEEK *et al* 1957) Total intracellular sodium can be estimated to amount to 0 4—0 5 meq in the size of rat concerned According to SKELTON (1927) 10 9 per cent of the rat body weight is bone This provides a means of calculating the total amount of bone sodium as well as the total amount of exchangeable bone sodium The available evidence (EDELMAN *et al* 1954 BERGSTROM and WALLACE 1954) indicates a fair degree of constancy from location to location with regard to sodium concentration in cortical bone The mean extracellular fluid volume based on such a calculation is 41 4 ml in untreated 186 1 g rats and in the rats on low sodium diet 34 5 and 36 3 ml respectively CHEEK *et al* (1957) using an entirely different technique found an extracellular sodium space of 47 9 ml and an extracellular chloride space of 47 4 ml in their 220 g standard rat The sodium loss following the low sodium diet occurs therfore mainly from the extracellular fluid and in the form of a reduction of the volume the sodium concentration of the fluid remaining constant In the rats treated with low sodium diet for three days the changes in the extracellular fluid account for 90 per cent of the lost sodium and the bone for 10 per cent In the rats treated for 5 days with this diet the extracellular fluid changes account for the complete loss of sodium

The weight loss in the sodium depleted groups almost certainly represented fluid loss In the 5 day rats the weight loss was 4 8 g and the reduction in extracellular volume 5 1 ml The corresponding figures for the rats on sodium restriction for 3 days were 4 3 g and 6 9 ml One explanation for this apparent

Table I *Results obtained in 200 g rats treated with low sodium diet only and low sodium diet in combination with acidosis the latter produced by intraperitoneal dialysis against an ammonium chloride solution*

Type of treatment	Group 1 Normal untreated 200 g control group (18 rats) Mean ± S.D	Group 2 Low Na diet 3 days (6 rats) Mean ± S.D	Group 3 Low Na diet 5 days (6 rats) Mean ± S.D	Group 4 Low Na diet 3 days + acidosis (10 rats) Mean ± S.D
Final weight (g)	186.1 ± 8.0	181.8 ± 7.5	181.3 ± 5.6	178.0 ± 5.6
Total exch. body Na (mEq)	9.03 ± 0.72	7.9 ± 0.32	8.14 ± 0.35	7.21 ± 0.78
Total exch. body K (mEq)	13.28 ± 1.2	12.41 ± 1.10	13.8 ± 1.0	12.5 ± 1.3
Urine volume (ml)	26.0 ± 26.0	12.6 ± 8.9	10.8 ± 7.3	21.5 ± 6.9
Urine Na (mEq)	0.81 ± 0.3	0.45 ± 0.22	0.27 ± 0.23	0.02 ± 0.02
Urine K (mEq)	0.90 ± 0.25	0.82 ± 0.29	0.[illegible]8 ± 0.27	0.9 ± 0.30
Total bone Na conc. (mEq/kg wet bone)	203.6 ± 13.1	197.8 ± 9.9	205.3 ± 16.0	189.7 ± 8.6
Exch. bone Na conc. (mEq/kg wet bone)	115.0 ± 8.8	110.5 ± 5.5	113.1 ± 9.9	103.8 ± 10.0
Total bone K conc. (mEq/kg wet bone)	26.0 ± 2.4	20.7 ± 2.22	[illegible]3 ± 2.95	21.8 ± 2.23
Exch. bone K conc. (mEq/kg wet bone)	25.7 ± 2.5	19.9 ± 1.17	27.5 ± 4.5	23.0 ± 3.7
Plasma Na conc. (mEq/l)	148.0 ± 1.6	149.0 ± 7.09	147.1 ± 2.4	14[illegible].1 ± 6.14
Plasma K conc. (mEq/l)	3.8 ± 0.60	4.03 ± 0.18	3.9 ± 0.51	4.16 ± 0.57

Urine volume during the equilibration period with radioisotopes in the metabolic cages

discrepancy may be that the loss of electrocytes from the extracellular space was associated with a movement of water into the cells

The finding of a reduced total body potassium a normal urinary potassium and a slightly elevated plasma potassium concentration in the rats fed on the low sodium diet for 3 days is in agreement with the observations of ANDERSON and LARAGH (1958) The sodium restriction does not hamper the renal ability to excrete potassium in Group 2 and 3 Table I The bones contribute relatively more than the other tissues to the potassium loss associated with the early phase of sodium restriction

BERGSTROM (1952) and NICHOLS and NICHOLS (1956) obtained a significant fall in bone sodium in their deprivation studies The sodium depletion however may have been of a more marked degree in their experiments since it was induced by mercurial diuretics and Diamox treatment In the experiments of MUNROE *et al* (1957) there was a considerable fall in the inexchangeable bone sodium fraction by diet alone

Table II Results obtained in 200 g rats on high sodium chloride intake for different periods of time

	Group 1 4 days (6 rats) Mean ± S D	Group 2 6 days (13 rats) Mean ± S D	Group 3 8 days (7 rats) Mean ± S D	Group 4 15 days (8 rats) Mean ± S D
Final weight (g)	190.0 ± 6.8	194.2 ± 9.3	202.1 ± 7.9	07.9 ± 10.3
Total exch body Na (mEq)	9.58 ± 0.66	8.81 ± 1.58	8.66 ± 1.07	9.3 ± 0.67
Total exch body K (mEq)	13.09 ± 1.13	13.29 ± 2.43	1 .09 ± 0.85	14.46 ± 1.6
Urine volume (ml)	95.1 ± 57.7	83.4 ± 54.7	81.0 ± 7.7	40.9 ± 41.0
Urine Na (mEq)	8.70 ± 5.08	6.9 ± 4.5	7.35 ± 4.81	3.17 ± 3.10
Urine K (mEq)	1.39 ± 0.34	1.17 ± 0.3	1.25 ± 0.41	0.74 ± 0.
Total bone Na conc. (mEq/kg wet bone)	187.2 ± 10.3	208.6 ± 16.5	215.2 ± 7.1	211.4 ± 31.5
Exch bone Na conc. (mEq/kg wet bone)	114.6 ± 9.4	113.0 ± 21.0	115.6 ± 10.4	115.1 ± 11.7
Total bone K conc. (mEq/kg wet bone)	23.1 ± 1.8	23.7 ± 2.8	23.3 ± 4.2	34.4 ± 3.9
Exch bone K conc (mEq/kg wet bone)	23.0 ± 2.0	23.5 ± 3.9	26.2 ± .8	20.9 ± 4.4
Plasma Na conc (mEq/l)	150.4 ± 3.3	148.6 ± 2.4	147.9 ± 2.7	148.5 3.0
Plasma K conc (mEq/l)	3.1 ± 0.34	3.4 ± 0.9	3.5 ± 0.3	3.0 ± 0.55

As Table I

Acidosis (Table I Group 4)

Groups 2 and 4 were identically treated apart from the induced acidosis Group 4 displayed an additional significant fall (P—0.05) in total body sodium when compared with Group 2 When compared with the normal control rats in Group 1 the difference was statistically significant at the P—0.01 level for the body weight the total body sodium and the total and exchangeable bone sodium concentrations The parallel fall in the total and exchangeable bone sodium (P—0.05) indicated that the sodium was lost from the exchangeable fraction The acidotic rats showed a marked reduction of sodium in their urine in spite of an increased urine volume and slightly increased urine potassium

A similar calculation for the acidosis group in Table I as employed above for the rats on low sodium diet gives an extracellular fluid volume of 31.7 ml. Group 2 in the same table has an extracellular fluid volume of 34.5 ml The induced acidosis has caused a loss of 0.69 meq of sodium from the rat body as can be seen by comparing the total exchangeable body sodium values The loss is made up of 1 a shrinkage of the extracellular volume of 2.8 ml with 0.417 meq sodium 2 a fall in extracellular sodium concentration which accounts for 0.135 meq and 3 a fall in exchangeable bone sodium concentration which accounts for 0.133 meq of sodium 19.3 per cent of the sodium loss from th

acidosis as such is therefore taken from the bones When the total reduction in body sodium resulting from the low sodium diet and acidosis is considered only 13 2 per cent is covered from this source Acidosis rather than pure sodium depletion therefore will remove sodium from the bone matrix in accordance with BERGSTROM's (1954) hypothesis of exchange with hydrogen ions at this location The size of the bone contribution in the presented acidosis experiments is more in agreement with that found by NICHOLS and NICHOLS (1953) than by BERGSTROM (1955)

Sodium chloride loading (Table II)

The urinary volume and the urinary sodium output from the last 15 hours indicates a very high sodium turnover induced by the procedure The rats thrived on the diet and they gained steadily in weight during the 15 days of treatment The greatest changes again occurred after the shortest period of treatment In Group 1 plasma sodium and total body sodium were elevated The plasma potassium was low in all of the groups in Table II but lowest in Group 1 It is most remarkable that the bone sodium concentration was reduced ($0.05 > P > 0.01$) in this group when compared to normal untreated controls Total bone sodium concentration was slightly increased in the other three groups the highest value was found in Group 3 (P—0 05 versus the untreated controls) A considerable variation in the concentration of the exchangeable bone sodium occurred in Group 2 In Group 4 the total bone potassium was markedly increased (P—0 01) in comparison with untreated rats

The results agree with earlier findings as regards the great capacity of the rat kidney to handle excess sodium (WESSON *et al* 1950 KELLOG BURACK and ISSELBACHER 1954) On the other hand the data also indicate that the mechanisms involved in the adaption of the rats to the abnormal salt intake need a certain time to be fully effective In Group 1 after 4 days of treatment there is a slight sodium retention as shown by the high total exchangeable body sodium the high plasma sodium and an extracellular volume of 44 0 ml (calculated as before) The low plasma potassium and total bone sodium concentrations indicate the strain on the mechanisms involved The finding of a relative reduction of the total body exchangeable sodium and of the extracellular spaces (39 4 and 37 6 ml respectively) in Groups 2 and 3 after 6 and 8 days of treatment may be interpreted as signs of overcompensation In the last group after 15 days of treatment body weight (207 9 g) total exchangeable body sodium and extracellular space (44 2 ml) are again in normal proportion Significantly in this group urine volume and sodium content are much smaller than in the former groups This again may be interpreted as representing an adaptation to the constant abnormal intake No edema was present The possibility also exists that the decreased sodium potassium and water output is caused by renal incapability in keeping up with the load No examination of the kidneys

Table III Results obtained following alkalosis induced by intraperitoneal dialysis against sodium bicarbonate in rats on standard diet and low potassium diet for 9 days

Type of treatment	Group 1 Standard diet + alkalosis (8 rats) Mean ± S D	Group 2 Low K diet (8 rats) Mean ± S D	Group 3 Low K diet + alkalosis (8 rats) Mean ± S D	Group 4 Low K diet 9 days 2 days Doca + alkal. (8 rats) Mean ± S D
Final weight (g)	195.1 ± 7.5	152.8 ± 7.9	153.8 ± 7.3	151.4 ± 4.8
Total exch body Na (mEq)	8.76 ± 0.37	6.99 ± 0.47	7.54 ± 0.76	7.67 ± 0.54
Total exch body K (mEq)	13.11 ± 1.29	9.88 ± 1.05	9.82 ± 1.65	9.85 ± 0.94
Urine volume (ml)[1]	8.7 ± 2.3	3.9 ± 2.2	10.2 ± 6.4	9.0 ± 6.8
Urine Na (mEq)	1.18 ± 0.26	0.42 ± 0.23	1.35 ± 0.28	1.06 ± 0.37
Urine K (mEq)	1.13 ± 0.20	0.23 ± 0.14	0.41 ± 0.22	0.43 ± 0.29
Total bone Na conc (mEq/kg wet bone)	214.2 ± 21.4	208.8 ± 10.2	211.2 ± 13.1	200.8 ± 19.7
Exch bone Na conc. (mEq/kg wet bone)	113.9 ± 6.9	106.7 ± 8.2	112.2 ± 6.5	110.8 ± 7.4
Total bone K conc. (mEq/kg wet bone)	24.2 ± 2.2	24.5 ± 2.7	24.3 ± 1.5	25.0 ± 3.6
Exch bone K conc (mEq/kg wet bone)	23.5 ± 2.5	20.9 ± 3.4	20.7 ± 5.1	22.4 ± 3.8
Plasma Na conc (mEq/l)	145.1 ± 2.3	148.8 ± 1.[illegible]	146.6 ± 3.9	146.9 ± [illegible]
Plasma K conc (mEq/l)	3.4 ± 0.8	3.4 ± 0.45	3.5 ± 0.43	3.3 ± [illegible]

[1] As Table I

for structural changes was performed The 4 to 5 per cent increase of sodium concentration in bone represents the extent of storage of this ion in this location. Nichols and Nichols (1956) found an 8 per cent gain in the total sodium with chronic sodium loading in the dog

Low potassium diet (Table III Group 2)

The rats did not thrive on this diet they huddled in the corners of the cage and finally almost stopped eating Their fur became dull and tattery An anorexic effect of a low potassium diet was observed by Smith *et al* (1960) There was a statistically significant fall in weight total body sodium and potassium (P—0 01) The other parameters did not display any marked variation from untreated rats A continued potassium loss took place in the urine throughout the experimental period the urinary potassium concentration being 59 meq l on the 9th day of potassium deprivation On the 6th day of sodium deprivation the urinary sodium was only 25 meq/l which again demonstrates the greater efficiency of the sodium preserving mechanisms

The measurement of the weight and the total exchangeable sodium and potassium spaces in these rats reveals a proportional relationship almost

Table IV Account of the e ternal sod um p tassium and chloride gains and losses induced by the intraperitoneal i jection of bicarbonate solution

	Mean values expressed in mEq		
	Gr up 1	Group 3	G oup 4
Sod m			
Injected intraperitoneally as NaHCO	6,410	4 830	4.830
Remov d in dialysate	2.764	1 806	1 778
N t gain to the rat body by d alysis	3 646	3 024	3 052
Recovered in the urine during th dialysis	2.561	1 910	1.879
Sodium left in th rat af r dialy is	1 085	1 114	1 175
P tassium			
Injected nt aperitoneally as KHCO	0 007	0 006	0 006
Remov d in dialysate	0 061	0 042	0 038
Rem ed fr m the rat body by d al is	0 0 4	0 036	0 032
Recovered in the urin *during* th dialysis	0 098	*0 062*	0 129
Negati e balan after *dialysis* potass um	0 152	0 098	0 161
Chlorid			
Removed n dialysate	1 482	0 976	0 971

identical to that of normal untreated rats This lends support to the view that the results obtained while representing potassium deprivation still to a large extent reflect an effect of the associated self induced starvation Furthermore this proportional decrease does not lend support to the vie v that sodium is substituted for potassium in muscles and other tissues during potassium depri vation (GAMBLE *et al* 1951 CHEEK and WEST 1956 ANDERSON and LARAGH 1958)

Alkalosis (Table III Groups 1 3 and 4 and Table IV)

As shown in Table IV the dialysis finally left about 1 1 meq of sodium in all types of rats in addition the alkalosis was aggra ated by the removal of chloride but relieved slightly by loss of potassium in the urine during the procedure This last effect was mainly present in Group 1 The net result was a displace ment in the direction of alkalosis of the same extent in all three groups of animals

Turning to Table III the great capac ty of the normal rat kidney to handle the acute sodium bicarbonate excess is e ident In Group 1 2/3 of the absorbed load is excreted during the 3 hours of dialysis and the remaining 1/3 during the follo ing 15 hours The excreted amount actually slightly exceeds the load The acid base imbalance caused by the chloride loss is fully compensated for by the potassium excretion Both the total exchangeable sodium and potassium

are slightly lower than normal indicating that the loss of chloride almost totally an extracellular ion is balanced by a reduction of cations from the intra and extracellular spaces

The increase in total sodium space in Group 3 and 4 when compared with *Group 2* the control group of this series indicates reduced efficiency in the handling of the alkalosis When the urinary sodium is compared with the amount entering the rat body a small sodium retention is demonstrable in Group 4 but not in Group 3 The potassium excretion is slightly larger in Group 4 than in Group 3 Both of these effects are probably caused by the injection of desoxycorticosterone in the rats of Group 4 In both groups the chloride loss is very far from being compensated The potassium depleted rats are still in a severe state of alkalosis at the end of the observation period confirmatory to earlier observations (COOKE *et al* 1954)

The reduced ability to excrete potassium in Group 3 does not depend upon a low plasma potassium the plasma potassium level being similar to that of the rats in Group 1 A non availability from the tissues in the potassium depleted rats may explain the falling excretion

Some storage of sodium may take place in the bones during alkalosis the changes seem to be confined to the inexchangeable fraction and to be influenced by desoxycorticosterone

The results have been collected in experiments that put severe strain on the electrolyte regulatory mechanism of the rat The acidosis when carried out with slightly larger doses of intraperitoneal dialysate or over longer periods than usually used resulted in a high mortality rate The same applied to the experiments with induced alkalosis The potassium depleted rats were moribund During the sodium loading the the sodium turnover in the rat organism per 24 hours by far exceeded the total body sodium content of the rat In spite of this the total bone sodium concentration varied only from 189 7 meq/kg in acidosis to 211 2 meq/kg in alkalosis. The difference of 21 5 meq/kg corresponds to 10 per cent of the total bone sodium The difference between the extremes mentioned above is calculated to be 0 47 meq or only 5 per cent of the total exchangeable sodium in the 200 g rat

The constancy of the bone potassium concentration is striking The variations in the bone sodium and potassium concentration do not run in parallel The experiments presented certainly indicate a much smaller importance of bone as a sodium and potassium reservoir than is often assumed

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The Participation of Bone in the Sodium and Potassium Metabolism of the Rat III

The effect of adrenalectomy, cortisone, desoxycorticosterone and 2 methyl 9 α fluorohydrocortisone

By

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Abstract

NORMAN N *The participation of bone in the sodium and potassium metabolism of the rat III* Acta physiol scand 1963 57 384—396 — Experiments were performed on the effect of sodium loading and sodium depletion in adrenalectomized rats These treatments were combined with injections of cortisone desoxycorticosterone acetate and 2 methyl 9-α fluorohydrocortisone in unoperated as well as adrenalectomized animals In the moribund stage after adrenalectomy when total body exchangeable sodium was reduced by more than 25 per cent only 6 per cent of the lost sodium was derived from bone The effect of the cortical compounds was found to be entirely dependent on, and varying with the particular state of the test animal, its electrolyte intake the length of time on the diet regimen and whether it was adrenalectomized or not Only slight contributions from the bone to the total body pool of sodium and potassium were noted in these experiments with the cortical compounds. Profound changes were however produced in the relationship between the exchangeable and inexchangeable fraction of the bone sodium by these compounds in combination with sodium loading of the animals. The observations are discussed in relation to present day concepts of the mechanism of sodium binding in bone and a possible connection to the pathology of collagen disease is pointed out

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In comparison with the very great number of publications on the effect of adrenalectomy and adrenal cortical compounds on the excretory aspects of sodium and potassium metabolism the studies directly concerned with the influence of these treatments on the bone electrolytes are few WHITE and ROLF (1954 1955) found a slight fall in bone sodium and a slight increase in potas sium after adrenalectomy Their bone samples included however the total skeleton with marrow pieces of tendon and spinal cord FLANAGAN and OVER MAN (1949) FLANAGAN DAVIS and OVERMAN (1950) and STERN *et al* (1951) assumed that sodium was deposited in bone after adrenalectomy on the basis of external balance studies in combination with extracellular space measure ments They were unable to confirm this by direct bone analysis WOODBURY (1953) could prevent sodium loss from bone which occurred in his rats during fasting by giving desoxycorticosterone No increase in bone sodium was observed in fed rats that were given the compound MUNROE SATOSKAR and WILSON (1958) were able to show that bone sodium decreased after adrenalectomy and low sodium diet the loss occurred from the exchangeable fraction

The relationship between the exchangeable and inexchangeable fractions of sodium and potassium in bone during the variations in electrolyte intake and treatment with cortical compounds has not been studied before The location of the two fractions in the ultrastructure of bone is not established HENDRICKS (EDELMAN JAMES and MOORE 1952) considering all excess bone sodium to be located on the surface of the apatite micro crystals suggested that the ex change was incomplete because of the inaccessibility of parts of these surfaces In this view he has been supported by NICHOLS and NICHOLS (1956) Considering the complex structure of the cortical bone substance with close linkage be tween crystals mucopolysaccharides and collagen fibres other possibilities certainly present themselves The study of chondroitin sulphate by FARBER and SCHUBERT (1957) is stimulating in this respect As will be shown some of the results obtained in the present study are difficult to explain on the basis of Hendricks hypothesis

Experimental Procedure

Methods

For methods and calculations the reader is referred to NORMAN (1963 a) Male rats of the hooded Royal Victoria Hospital strain weighing between 198 and 202 g were used throughout.

Diets

The experimental diets have been described elsewhere (NORMAN 1963 b)

Adrenalectomy

Both adrenals were removed in one stage under ether anesthesia and care was taken to remove them within their capsules In the postoperative period the rats always received 0 9 per cent sodium chloride *ad libitum* as the drinking fluid Dietary regimes wer

Table I Results obtained in ?00 g rats after 5 days on the lou sod um diet combined uith cortisone desoxycorticosterone acetate (Doca) and 2 methyl 9 α fluorohydroco tiso e (2 M9αF) treatment

Type of treatment	Group 1 Low Na d et only (6 rats) Mean ± S D	G oup 2 Low Na diet + cortisone (6 rats) Mean ± S D	Group 3 Low Na d et + Doca (8 ats) M an ± S D	G up 4 Low Na d et + ?M9 F (6 rats) Mean ± S D
F nal weight (g)	181 3 ± 5 6	173.5 ± 12 5	181 0 ±) 1	188 1 ± 10 4
Total exch body Na (mEq)	8 14 ± 0 85	7 46 ± 0 43	8 3› ± 0 89	8 4 ± 0 3
Total exch body K (mEq)	13 80 ± 1 00	12 19 ± 0 74	13 75 ± 1 14	13.25 ± 1 42
Ur ne volume (ml)	10 8 ± 7 3	11 2 ± 5 1	12.8 ± 3 7	6 7 ± 1 9
Urine Na (mEq)	0 27 ± 0 23	0 09 ± 0 10	0 32 ± 0.21	0 36 ± 0.29
Urine K (mEq)	0 78 ± 0 27	0 68 ± 0.20	0 /8 ± 0 16	0 64 ± 0.31
Total bc ne Na conc (mEq/ kg et bone)	205 3 ± 16 0	198 7 ± 11 96	19 0 ± 9 6	202 0 ± 6.9
Exch. bone Na conc. (m Eq/ kg et bone)	113 1 ± 9 9	118.0 ± 10 3	100.4 ± 4 7	111 0 ± 9 6
Total bone K con (mEq/ kg wet bone)	24 3 ± 2 9›	24 3 ± 3 68	6 7 ± 6 64	22 6 ± 1 1
Excl bone K conc (mEq/ 1 g wet bone)	27 5 ± 4 5	24 2 ± 2 7	27 8 ± 6 3	2? 8 ± .6
Pla ma Na conc (mEq/l)	147 1 ± 2 4	147 4 ± 3 5	147 5 ± 1 8	148.3 ± 3.3
Plasma K conc (mEq/l)	3 9 ± 0 ›1	4 2 ± 0 50	3 6 ± 0 37	4 1 ± 0.57

Urin volume during th last 15 hours

instituted 24 hours after the operation The hormone treatments were started on the dav of operation

Hormones

The hormone preparations were injected every day subcutaneously as follo s Cortisone (Cortone Merck) 1 25 mg morning and evening Desoxycort osterone acetate in sesame oil (Ciba) 0 5 mg in the morning 2 methyl 9-α fluorohydrocortisone (Upjohn Company) 0 01 mg in propyleneglycol-ethylalcohol morning and evening

Results

The effect of low sodium diet and adrenal cortical compounds in intact rats (Table I)

Group 1 received the low sodium diet only and it serves as a control It should be recalled however that such rats have a reduced body weight and total exchangeable sodium compared with rats on standard diet (Table III *Group 1*)

Cortisone but not the two other steroids induced a fall in body weight total exchangeable body sodium and potassium (0 01 > P < 0 05) *Doca* caused a significant fall in total bone sodium (P < 0 01) *The effect of cortisone*

Table II Results obtained in 200 g rats after 6 days (group 1 2 3 4) and 15 days (group 5) on a high sodium intake combined with cortisone desoxycorticosterone acetate (Doca) and 2 methyl 9 α fluorohydrocortisone (2M9αF) treatment

Type f treatment	G oup 1 St nd rd d et + N Cl (13 rats) Mean ± S D	Group 2 Stand rd di t + NaCl + n ne (6 rats) M an ± S.D	G oup 3 Standa d diet + NaCl + Doca (12 rats) Mean ± S D	G oup 4 Standard d et + NaCl + 2M9αF (7 s) M n ± S D	Group 5 Standa d diet + NaCl + Doca (7 rats) Mean ± S D
Final we ght (g)	194 2 ± 9.3	181 2 ± 9 6	189 ± 10 7	197 7 ± 7 4	204 5 ± 7 5
T tal exch body N (mEq)	8 81 ± 1 8	7 99 ± 1 61	8.48 ± 1 13	10..6 ± 2 46	9 85 ± 0 56
T tal exch body K (mEq)	13 29 ± 2 43	11 78 ± 0 93	13 39 ± 1 61	13 41 ± 2 06	15.22 ± 1 6
Urin v lume (ml)	83 4 ± 54 7	96 9 ± 59 0	53 4 ± 49 9	77 2 ± 24 0	88.1 ± 56 6
U in Na (mEq)	6 9 ± 4 5	8 07 ± 5 3	4 8 ± 3 8	7 6 ± 3 4	6.19 ± 4 04
Uri e K (mEq)	1 17 ± 0 30	1 02 ± 0 27	1 1 ± 0 43	1.24 ± 0.27	0 92 ± 0 34
T tal bon Na conc (mEq/kg wet bone)	208 6 ± 16 5	194 4 ± 7 3	19 9 ± 5 5	210 7 ± 5 3	218.5 ± 7 04
Exch bon N con (mEq/kg w t bone)	113 0 ± 21 0	103 0 ± 8 9	136.2 ± 35 5	115 6 ± 11 0	113 8 ± 9 33
T tal bon K conc (mEq/kg w t bon)	23 7 ± 2 83	23 8 ± 1 41	23 3 ± 4.26	21 1 ± 2 94	17 1 ± 2.32
Exch bon K on (mEq/kg w t bon)	23 5 ± 3 9	23 3 ± 2 43	23 6 ± 3 77	21 2 ± 2 9	18 0 ± 2 99
Plasma Na conc (mEq/l)	148 6 ± 2 4	14 0 ± 3 4	146.6 ± 4 9	149 2 ± 2.4	149 5 ± 1 89
Plasm K n (mEq/l)	3 4 ± 0 92	4 2 ± 0 63	3 01 ± 0 51	2 7 ± 0.27	2 4 ± 0 36

Urin luta during th las 15 hours

and Doca on the exchangeable bone sodium differed statistically (P—0 01) Bone and plasma sodium and potassium did not otherw ise deviate from the values in untreated animals

The extracellular fluid volume as by calculation (NORMAN 1963 b) 36 3 ml in Group 1 and 31 4 ml in Group 2 the latter having received cortisone The respecti e eights of the rats in these groups were 181 3 g and 173 5 g The weight lo s as therefore in th s instance not completely accounted for by the shrinkage of extracellular fluid volume tissue mass as well must have been reduced in the cortisone treated group The fall in total potassium in these animals supports th s contention

The effect of sodium loading and cortical compounds in intact rats (Table II)

All rats in this series were thriving during the experimental period

Comparison of the data from the groups of rats listed in Table II with those of untreated animals (Table III Group 1) revealed no statistically significant deviation in the group receiving the sodium chloride loading only The added cortisone treatment gave a significant fall in the total exchangeable potassium (P—0 01) the 6 day Doca treatment caused an increase in the exchangeable bone sodium (0 05 > P > 0 01) and the 9 α fluorohydrocortisone produced a fall in plasma potassium (P—0 01) and an increase in the total exchangeable body sodium (0 1 > P > 0 05)

The separate effects of the 6 day administration of corticosteroids during the loading experiment can be evaluated by comparison of Groups 2 3 and 4 with Group 1 in Table II

The most marked changes followed the cortisone treatment which caused a fall in body weight (0 05 > P > 0 01) a fall in total bone sodium (0 5 > P > > 0 01) a fall in plasma sodium (0 05 > P > 0 01) and an increase in plasma potassium (0 1 > P > 0 05) The reduction in the total exchangeable body sodium and potassium was not statistically significant

Doca treatment resulted in a reduction in the total bone sodium (0 05 > P > > 0 01) The results in Group 3 and 4 otherwise differed very little from those of Group 1 The elevated exchangeable sodium in the bone in Group 2 although striking is not statistically significant

The body weight total body sodium and potassium increased significantly in Group 5 (Doca + NaCl 15 days) The proportion between these parameters was the same as in untreated rats and it is probable that the observed increments represented normal growth The significant fall in bone *potassium* (P—0 01) might be interpreted the same way as the bone potassium *tends to* fall with age (NORMAN 1963 a) The increased total bone sodium (P—0 01) the low plasma potassium (P—0 01) and the high plasma sodium definitely resulted from the treatment

The effect of low sodium diet and adrenal cortical compounds in adrenalectomized rats (Table III)

The rats on the low sodium diet after adrenalectomy kept well and ate normally for the first few days From the 5th day postoperatively they deteriorated rapidly stopped eating and huddled in the corners of the cage without moving more than 50 per cent of them died on the 6th and 7th day *Group 3* is a collection of such rats Smaller amounts of blood than usual were obtained from these and the hematocrit value was visibly increased The treatment with Doca and 9 α fluorohydrocortisone kept the animals close to normal in the period of observation Cortisone on the other hand was clearly less adequate These rats were weak and in poor condition

The main effect of adrenalectomy and low sodium diet was a marked fall in total body sodium and an increase in plasma potassium (P—0 01) occurring as early as the 3rd postoperative day Two days later the changes were still more marked and additionally a fall in body weight (P—0 01) in plasma sodium (P—0 01) and in exchangeable bone sodium (P—0 01) was found The total bone sodium and body potassium were not significantly below the normal range The total sodium loss amounted to 2 41 meq and the weight loss to 19 5 g in the course of the five days when compared to the normal 186 1 g rat The calculated extracellular fluid volume was 41 4 ml and 30 2 ml respectively The various body compartment contributed to the loss as follows shrinkage of extracellular fluid volume 75 per cent fall in extracellular sodium concentration 19 per cent and loss of sodium from bone 6 per cent

The addition of cortisone during the experimental period still left the animals with a reduction in body weight (P—0 01) and total body sodium (P—0 05) as well as an elevated plasma potassium value (P—0 05) The total body potassium was reduced (P—0 05) Doca maintained body weight total body sodium and potassium at normal levels but the plasma potassium remained elevated (P—0 05) *In these rats there was a significant increase in both total* and exchangeable bone sodium (P—0 05)

Finally the 9 α fluorohydrocortisone kept the weight and plasma potassium concentration within the normal range but the total exchangeable body potassium (P—0 01) and sodium (0 05 > P > 0 01) were reduced as was the total bone potassium Both total and exchangeable bone sodium were below normal (P—0 1 and P—0 01 respectively)

Series of sham operated animals received the low sodium diet The data from these did not differ from those of unoperated groups

The effect of sodium loading and adrenal cortical compounds in adrenalectomized rats (Table IV)

The rats in Groups 1 2 and 3 behaved normally until the time of examination The results show that there was a temporary set back following the adrenalectomy as demonstrated by a reduced total exchangeable body sodium (P—0 01) and potassium (0 05 > P > 0 01) at 4 days the measurements returned to the normal range at 6 and 8 days The only exception to this being the slightly elevated plasma potassium and the low plasma sodium concentration (P—0 01) The exchangeable sodium in bone varied considerably in the rats after 6 and 8 days of treatment

In group 4 kept on sodium chloride and glucose for 15 days 6 of the original 8 rats started a down hill course 8 to 10 days postoperatively and one of them died The data in Table IV are from the surviving 5 rats The moribund state of these animals is reflected by a very low weight (P—0 01) total body potassium (P—0 01) and sodium (P—0 01) The plasma potassium was not significantly elevated but the plasma sodium was far below normal (P—0 01) T

Table III Results obtained in 200 g adrenalectomized rats postoperatively given the low sodium cortisone (2M9aF) treatment

Type of treatment	Group 1 Untreated intact (18 rats) Mean ± S D	Group 2 Adrenalectomy + low Na diet 3 days (8 rats) Mean ± S D
Final weight (g)	186 1 ± 8 0	1/8 9 ± 8 2
Total exch body Na (mEq)	9 03 ± 0 72	7 65 ± 0 3
Total exch body K (mEq)	113.28 ± 1 2	13 37 ± 1 69
Urine volume (ml)	36 0 ± 26 0	8.9 ± 7 8
Urine Na (mEq)	0 81 ± 0 30	0 36 ± 0 55
Urine K (mEq)	0 90 ± 0 25	0 75 ± 0.26
Total bone Na conc (mEq/kg wet bone)	203 6 ± 13 1	196 2 ± 15 9
Exch bone Na conc (mEq/kg wet bone)	115 0 ± 8.8	112 0 ± 7 4
Total bone K conc. (mEq/kg wet bone)	26 0 ± 2 4	30 7 ± 4 91
Exch bone K conc (mEq/kg wet bone)	2 7 ± 2	25 1 ± 4 18
Plasma Na conc. (mEq/l)	148 0 ± 1 6	147 6 ± 4 ?
Plasma K conc (mEq/l)	3 8 ± 0 60	4 8 ± 0 48

Urine volume during the last 15 hours.

duction in exchangeable bone sodium and total bone potassium concentration was significant (P—0 01) The mean value of the total bone sodium is the lowest observed during the experiments but because of the variation in results only of borderline significance (0 1 > P > 0 05) Calculation on the basis of the mean value indicated that the fall in bone sodium concentration accounted for close to 20 per cent of the total sodium loss The remaining 2 rats in Group 1 continued to thrive and at examination after 15 days of treatment showed entirely normal results No accessory adrenal tissue was found at autopsy

Of the corticosteroids cortisone had the most marked effect In Group 5 the total exchangeable body sodium was reduced to the same level as seen in adrenalectomized rats receiving no supportive therapy The urine volume and the sodium excretion were much higher than in any of the other adrenalectomized rats and significantly different (1—0 01) from those in Group 2 that were similarly treated but received no cortisone The other parameters in Group 5 were within the normal range

Doca administration resulted in increased weight total body sodium and potassium (P—0 01) when compared to Group 2 Total bone sodium was increased (0 05 > I > 0 01) and exchangeable bone sodium reduced (1—0 01) The plasma electrolytes were maintained at a normal level

9 α fluorohydrocortisone caused a profound fall in total exchangeable body potassium (P—0 01) in plasma potassium (P—0 05) and both total and ex

diet and add tionally cortisone desoxy o ticostero e acetate (Doca) and 2 methyl 9 α fluorohyd o

Group 3 Adrenalect my + low ∖a diet 5 days (11 rats) Mean ± S D	G oup 4 Adrenal ctomy + low N di t + ortison 5 d ys (7 ats) Me n ± S D	Gr up 5 Ad enalect my + lo v ∖a di t + Doca 5 day (6 rats) Mean ± S D	Group 6 Adrenalect my + l v ∖a di t + 2M9αF 5 days (6 r ts) Mean ± S D
166.6 ± 5 1	174 3 ± 8.3	189 2 ± 3 03	184 8 ± 4.8
6 62 ± 0 65	7 38 ± 0 89	8.95 ± 0 49	8.27 ± 0.22
12.36 ± 2.29	11 8 ± 1 33	14 07 ± 0 68	11 08 ± 1 11
7 0 ± 10 4	12 5 ± 11 8	3 3 ± 1 85	3 6 ± 1 1
0 11 ± 0 12	0 24 ± 0 20	0 22 ± 0 08	0 12 ± 0 04
0 36 ± 0 21	0 8 ± 0 20	0 4 ± 0 21	0 6? ± 0 04
196 6 ± 18 0	203 1 ± 13 25	215.2 ± 10 62	192 1 ± 14 1
97 7 ± 11 5	106 9 ± 10 97	114 1 ± 6 8	103.5 ± 6.4
32 8 ± 13 5	27 6 ± 3 09	2 9 ± 1 41	20 5 ± 2.12
26 6 ± 4 97	24 4 ± 2 62	26 8 ± 3 95	21.3 ± 1 62
137 9 ± 7 0	147 1 ± 4 9	150 2 ± 3 4	145.5 ± 0 51
5 5 ± 1.32	4 7 ± 0 8	4 5 ± 0 41	4 04 ± 0 57

changeable bone potassium (P—0 01) The weight of the rats was normal but total exchangeable body sodium was elevated (P—0 01) Total bone sodium was slightly low but only the exchangeable fraction significantly so (P—0 01)

Series of sham operated rats received the sodium chloride and glucose treatment The data from these did not differ from those of unoperated animals

Discussion

In the following the results from the experiments here presented as well as some of the results in the papers of NORMAN (1963 a b) are discussed

The total body weight and electrolyte changes observed after adrenalectomy and low sodium diet (Table III Group 3) correspond very closely to those found by WHITE and ROLF (1955) In their rats body potassium was reduced by 6 6 per cent body weight by 14 per cent and body sodium by 22 1 per cent The bone results are not directly comparable since they used bones including marrow spinal cord and periosteum They found a 4 5 per cent decrease in sodium and a slight increase in potassium results which also agree fairly well with ours Bone thus contributes little in compensating for the severe sodium loss in acute adrenal insufficiency The results obtained when treatment with sodium chloride and glucose maintained the rats for 15 days (Table IV Group 4) indicate that the release of sodium from bone may be greater with prolongation of the time interval up to the final state of adrenal insufficiency

Doca increased bone sodium in three of the experimental situations (Ta

Table IV Results obtained in 200 g adrenalectomi ed rats postoperat vely on a high sod um intake (2M9aF)

All rats were adrenalectomized

Type of treatment	Grou; 1 Standard diet + N Cl 4 days (7 rats) Mean ± S D	Group 2 Standard diet + NaCl 6 days (20 rats) Mean ± S D	Group 3 Standard diet + NaCl 8 days (8 rats) Mean ± S. D
Final weight (g)	180 0 ± 9 9	182 0 ± 9 7	190 0 ± 14 6
Total exch. bod; Na (mEq)	7 80 ± 0 66	8 93 ± 0 84	8 60 ± 1 36
Total exch. bod; K (mEq)	11.86 ± 0 89	13 00 ± 1 65	13 59 ± 1 0
Ur ne volume (ml)	8 8 ± 8 0	20 3 ± 15 2	38.8 ± 31 4
Urine Na (mEq)	1 3 ± 1 1	2 5 ± 1 3	3 71 ± 2.71
Urine K (mEq)	0 57 ± 0.23	0 68 ± 0 14	0 77 ± 0 38
Total bone Na conc (mEq/kg wet bone)	202.2 ± 16 4	206 7 ± 12.1	201 6 ± 3
E ch. bone Na conc. (mEq/kg wet bone)	106 7 ± 9 4	117 8 ± 30 6	102.7 ± 27 0
Total bone K conc. (mEq/kg wet bon)	2 9 ± 2 07	23 1 ± 3 7	2 3 ± 4 15
Exch. bone K conc (mEq/kg w t bon)	8 0 ± 3 47	23 9 ± 3 9	25 1 ± 6
Plasma Na conc. (mEq/l)	142 4 ± 11 8	142 4 ± 3 7	141 2 ± 5 9
Plasma K con (mEq/l)	4 3 ± 0.26	4 2 ± 0 0	4 2 ± 0 47

Urine volume dur ng the la t 15 hours.

II Group 5 Table III Group 5 Table IV Group 6) No increment exceeded 6 to 7 per cent of the normal value but even so this was higher than during sodium loading and alkalosis

Our experiments emphasize the stability of the total bone sodium concentration rather than the importance of bone as an available source of sodium in the course of extreme changes in the body content of this electrolyte

In normal intact rats as well as in all experimental groups receiving a low sodium diet the proportion between the exchangeable and the inexchangeable bone sodium varied very little When the rats received the sodium chloride and glucose solution in the drinking water however the constant proportion between the exchangeable and inexchangeable bone sodium fractions was severely disturbed as illustrated by the large standard deviations of the exchangeable bone sodium concentrations in these groups (Table II Group 1 Table IV Groups 2 and 3) The greatest variation was found in the adrenalectomized animals In Group 2 Table IV the exchangeable bone sodium varied from 35 per cent to 90 per cent in the normal rats between 5 and 60 per cent This variability following sodium loading was most marked after 6 and 8 days of treatment in both intact and adrenalectomized animals It was also

comb ned with cortisone desoxycorticosterone acetate (Do a) and 2 methyl 9 α fluorohydrocortisone

Gr up 4 Standard d et + N Cl 15 days (5 ats) Mean ± S D	Group 5 St ndard d et + NaCl + c tisone 6 days (6 rats) M an ± S D	Group 6 Standard d et + NaCl + Doca 6 days (7 rats) Mean ± S D	Group 7 Standa d d et + NaCl + 2M9αF 6 days (7 rats) Mean ± S D
161 2 ± 9	18 5 ± 2 2	199 9 ± 8 3	191 7 ± 7 8
7 24 ± 0 08	6 61 ± 1 51	10 09 ± 0 79	10 04 ± 0 55
11 02 ± 1 61	12 67 ± 0 88	14 99 ± 1 6	1 00 ± 1 56
10 6 ± 7 8	86 4 ± 53 9	38 5 ± 36 3	32 1 ± 31 2
1 48 ± 0 61	7 41 ± 4 5	3 60 ± 1 01	2.8 ± 2 32
0 62 ± 0 13	1 15 ± 0 7	1 29 ± 0 84	0 68 ± 0.21
186 2 ± 26 7	203 4 ± 3 7	210 5 ± 15 1	194 2 ± 9 16
103.0 ± 6 3	118 8 ± 10 7	91 5 ± 13 65	102 3 ± 69
19 5 ± 3 16	25 9 ± 1 82	24 8 ± 2 03	19 8 ± 2.2
21.8 ± 2 30	24 6 ± 1.2	26 1 + 3.23	21 1 ± 3 38
134.9 ± 4 5	148 4 ± 3 7	149 9 ± 5 26	147 6 ± 3 02
4 2 ± 0 34	4 4 ± 0 96	4 1 ± 0 7	3 1 ± 0 62

fluenced by the corticosteroids Cortisone maintained the normal variability and a normal proportion between the two bone sodium fractions (Table II Group 2 Table IV Group 5) Doca greatly increased the variability and the exchangeability in intact rats (Table II Gr up 3) and reduced the exchangeabil ity in adrenalectomized rats (Table IV Group 6) With prolonged treatment in normal rats (Table II Gr up 5) the exchangeability returned to normal 9 α flu rohydrocortisone (Table II Group 4 Table IV Group 7) maintained the uniformity of the group response but reduced the exchangeability in the adrenalectomized rats

The sodium loading experiments caused a tremendous turn over of sodium in the rat body as ho n by the urinary excretion values Careful examination revealed that no technical error or unreliable plasma specific activity measure ments resulted from this The fact that the cortisone treated animals in which the volume of urine and the excretion of sodium were highest maintained the most normal relationship between the t o bone sodium fractions supports this contention

The exchangeability which reflects the physico-chemical binding of sodium in the ultrastructure of cortical bor e is influenced by the intake of sodiu

chloride and by corticosteroids It is difficult to fit this observation into the current hypothesis for sodium binding in bone According to this bone sodium is located in the surface layer of the flat hydroxy apatite micro crystals of bone in part as the exchangeable fraction between the crystals The exchangeable fraction is considered to be identical with the metabolically active bone sodium (EDELMAN JAMES and MOORE 1952 FOLLIS 1952 NEUMAN TORIBARA and MULRYAN 1953 DALLEMAGNE and FABRY 1956)

Other possibilities do however present themselves Part of the organic bone matrix consists of mucopolysaccharides (FOLLIS 1952 MEYER 1956) These polymeric compounds have a great capacity for binding cations in a resin like fashion and may be involved in the storage of excess sodium (FARBER and SCHUBERT 1957) They furthermore have the ability to change the shape of the molecule when in solution The long chains fold and unfold with variations in the ionic environment (MARK and TOBOLSKY 1950 a b MEYER 1950 FLORY 1953) A close linkage amounting to actual chemical bonding has been demonstrated between the sulphur atoms of the mucopolysaccharides and the bone crystals and the collagen fibres (NEUMAN *et al* 1953 DALLEMAGNE and FABRY 1956) Mucopolysaccharides attached to the crystal surfaces may prevent or allow exchange of sodium ions in the outer layers of the crystals by creating or dissolving positively charged repelling shells This hypothesis with all of its imperfections may more easily explain the peculiar features observed in bone sodium exchangeability resulting from the hormonal stimuli than the rather rigid inorganic hypothesis mentioned before

The implication is that the cortical hormones act on the exchangeability of the bone sodium via an action on the mucopolysaccharides of the ground substance This may reflect on certain clinical and experimental observations in collagen disease Experimental collagen disease can be produced in rats by the administration of Doca and salt and prevented by cortisone In the experiments here presented Doca and sodium chloride destroyed the normal balance and created an abnormal ratio between the exchangeable and inexchangeable bone sodium fractions whereas cortisone normalized the response and the ratio This may reflect an action of these hormones at the molecular level and be connected with the basic structural pathology of collagen diseases

A significant inexchangeable bone potassium fraction was observed in only three instances 1 100 g normal rats 2 200 g normal rats treated with sodium chloride for 15 days 3 Adrenalectomized rats on low sodium intake In all other circumstances the bone potassium was completely exchangeable No relationship was demonstrated between the bone sodium and the bone potassium concentration changes This suggests a completely different location and binding of the potassium as compared with the sodium There was no constant connection between the total body potassium changes and the variations in bone potassium concentration The most constant positive correlation to the bone potassium concentration was found in the plasma potassium concentration

The adrenalectomized rats that received no supportive treatment died on the 6th and 7th day postoperatively When the rats were given the salt and glucose treatment after adrenalectomy for a prolonged period 6 rats out of 8 were dying or dead 15 days postoperatively Two of the 8 rats behaved and developed normally and it is probable that accessory adrenal tissue developed in these although no such tissue was found at autopsy These observations agree fairly well with those of GAUNT and GAUNT (1933—34)

Cortisone was sodium excretory as judged by the total body exchangeable sodium except in the case of adrenalectomized rats on low sodium diet in which a slight sodium retention was seen Doca maintained or increased the total bone sodium concentration in adrenalectomized rats on high and low sodium intake but intact rats lost sodium from the bone irrespective of the intake of the ion during Doca treatment These examples illustrate the fact that the same corticosteroid often gave inconsistent responses the direction of action being dependent on the physiological or pathophysiological state of the animal at the time of the steroid administration

It is generally agreed that the renal ability to excrete sodium and water is stimulated by cortisone (EVERSOLE GAUNT and KENDALL 1942 SALA and LUETSCHER 1954) The results presented in this study amply support this view the cortisone treated rats have a higher sodium and water turn over than any of the other groups Cortisone consistently caused a fall in the total body potassium in the intact animals the compound also produced a weight loss Plasma potassium was normal or high in these animals The evidence thus pointed to an antianabolic effect of the steroid

Desoxycorticosterone acetate (Doca) appeared to be the compound that kept the rats at the highest state of well being after adrenalectomy In the rats treated with Doca after operation the determined parameters were closest to normal There was a potassium retaining as well as a sodium retaining effect as judged by the total exchangeable spaces of these ions (Table III and IV) In contrast the plasma potassium level was very low The effect of the hormone seemed to be on the distribution of the potassium between the extracellular and intracellular compartment This was observed most markedly in Table II Group 5 in which the bone potassium was very low pointing to a connection between this compartment and the extracellular space

2 methyl 9 α fluorohydrocortisone (2M9αF) in most instances tended to maintain the sodium space but to reduce the potassium space In this respect it had some similarity to cortisone The plasma potassium level was however low in the case of 2M9αF whereas it was normal or high in the case of cortisone treatment This suggests that with regard to potassium metabolism 2M9αF had its primary effect on the renal excretory mechanism rather than being antianabolic

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The Splanchnic Hematocrit in Man

By

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Abstract

LARSEN O A N TYG TRUP and K WINKLER *The splanchn c hematocrit in man* Acta physiol scand 1963 *57* 397—406 — The aim of the present work as to study to what extent the difference be tween the total body hematocrit and the hematocrit of the blood in the large vessels is caused by a low h matocrit in the splanchnic area

I labelled albumin and Cr labelled red cells were njected into an antecub tal vein and samples were drawn simultaneously from the femoral artery and a h patic ein Th plasma and red cell olumes of the body and the body hematocrit were d termined from the acti ty inj cted and th calculated initial concentrations. The splanchnic hematocrit was calculated from th arterio-h pat c venous activity differences Th hepat c plasma flow as det rmined by the b omsulfalein m thod for calculat on of the planchnic plasma and red cell volumes In 10 normal subjects the planchnic h matocrit was not s gnificantly different from th body hematocrit The dif fe n e betw e n large essel hematocrit and body or splanchnic hematocrit may be expressed as an amount of extra plasma which in the bod on th a erag was 450 ml, and in the splanchnic area 105 ml Th results ndicate that th extra plasma is evenly distrib- uted throughout the maller essels in the body or that a substantial amount of extra plasma exists in some parts of the body not ex amined so fa

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The hematocrit value indicates the volume of red cells in per cent of the total volume of a given blood sample and is usually determined by centrifugation of stabilized blood from a peripheral vein in a graded tube. Various methods are available for determinations of the total red cell volume and the total plasma volume in the organism (GREGERSEN and RAWSON 1959). It is well established that the hematocrit value of the total body calculated from the most reliable of these determinations is about 10 per cent lower than the hematocrit determined in a blood sample from a large vessel (HEVESY et al. 1944, *GREGERSEN and RAWSON 1959*). *Two explanations are generally considered* to account for this discrepancy. 1) The hematocrit of the total blood (contained by definition in the cardiovascular system) may actually be lower than that of the blood in the larger vessels due to preferential accumulation of the red blood cells in the more rapidly moving axial stream of the smaller vessels (FÅHRÆUS 1929) and to the occurrence of capillaries containing plasma but no red cells. 2) The indicator used for total plasma volume determinations may leave this volume at a sufficiently high rate to make the calculated values erroneously high.

The findings that some organs, *i. e.* the lungs (LILIENFIELD *et al.* 1956, *PARRISH, STRANDNESS and BELL 1961*) and the brain (*ANDREE LARSEN and* LASSEN 1962) have hematocrit values greater than that of the total body indicate that the axial stream phenomenon is not the only cause to the discrepancy between body and large vessel hematocrit. It is therefore possible that the second hypothesis may explain a major part of the discrepancy.

As extravasation of plasma protein probably is more important in some organs depending of the structure of the capillaries, determinations of the hematocrit in different organs might clarify the question. In the work presented the hematocrit of the splanchnic circulation in man was investigated since the structure of the hepatic sinusoids probably permits interchange of intra and extravascular albumin (BENNETT, LUFT and HAMILTON 1959).

Methods

Ten subjects without signs of hepatic, circulatory or [illegible] were examined. Their age, sex, body weight and surface area (according to [illegible]) are given in Table I. They were kept fasting for [illegible] hours before the examination which took place in the morning, and they were premedicated with 200 mg [illegible] obarbit[illegible].

A LEHMAN catheter no. 9 with lateral opening [illegible] from the closed tip was placed in one of the right hepatic veins, and a polyethylene catheter [illegible] the same [illegible] as the venous catheter was placed in the femoral artery. For the determination of the hepatic blood flow by the method of BRADLEY *et al.* (1945) an infusion of [illegible] (5 mg/min) was started following a priming dose of 150 mg. [illegible] minutes later three blood samples were simultaneously drawn at intervals of four minutes from the two catheters for determination of bromsulfalein.

Labelled albumin and red cells contained in a volume of about [illegible] was then injected into an antecubital vein in the course of less than two seco[illegible]

T ble I

Case no.	Age, years	Sex	Body we ght kg	Body surfa m	MCT plasma s c	MCT red lls, sec	MCT plasma / MCT red cells
1	22	♀	53	1.5	22 8	19.8	1 15
2	15	♀	49	1 71	45.2	35 9	1.25
3	17	♀	63	1 6	58.2	45 3	1.29
4	33	♂	0	1.80	54 8	39 8	1 38
5	44	♂	66	1 79	49 0	37.3	1.3?
6	29	♀	73	1 81	27 6	25.9	1 08
7	19	♀	57	1 63	50 4	45 9	1 09
8	22	♂	70	1 78	40.3	36 6	1 08
9	45	♀	47	1 48	51 0	39 5	1.28
10	57	♂	6	1 85	42.9	39 4	1 10
		A erage	62	1 2	44.2	36.5	1.20
	St ndard deviation		—	—	11.5	8.3	13

series of samples of 2 5 ml each were drawn simultaneously from the two catheters by means of an automatic sampling apparatus Thirty pairs of samples were collected in the course of th ee minutes starting at the tim of the inj ction Thereupon sampling for determ nat on of bromsulfalein as repeated as above except in cases no 3 and 7 Fifteen minutes after the njection of the isotopes one arterial blood sample was dra n for determination of total body and large vessel hematocrit

Analytical procedures

The evening b fore the experiment the ed cells from 8 ml of the subjects blood were labelled w th Na ⁵ C O (GRAY and STERLING 1950) and after three washings with saline the supernatant contained no measurable radioactivity Human serum albumin was lab lled with I by the m thod of McFARLANE (1958) th amount of free iodine in the p eparat on b ing alw ys lower than one per cent of the total Immed ately befo e the expe ment about 120 μ of labelled red cells and 30 μc of labelled albumin were mixed

The activity of the ed blood c lls was determined in the cells from one ml of hepar inized whole blood The lls w e freed from plasma by washing w th saline until the supernatant was free from a tivity and saline was then added to a total olum of one ml The acti ity of plasma as determined as th difference betw een the activity in one ml of whole blood and that of th red cells Each sample was counted twice to 10 000 counts in a well scintillat on ounter using a on entional scaler ^{51}Cr counts per sample per minute g ne ally were about 10 times background and the total counts per sample per minute generally about 30 times background

The larg essel h matocr t was d termined by entrifugation of heparinized arterial blood in a mod fied W nthr be tube (3 000 p.m. for 30 min with a distanc of 15 cm from the ent of the ntrifug to the middle of the column of blood cells) A correction for trapped plasma was made by multiply ng the result by the factor 0 96 which was determined separat ly f th m thod and apparatus employed for the

determination using [131]I labelled albumin as an indicator for plasma (BRYDE ANDERSEN 1960). Bromsulfalein was determined by GAEBLER's method (1945). On average 150 ml blood was taken during the experiment.

Calculations

The hematocrit value (Htc) is the ratio between the red cell volume and the blood volume

$$\text{Htc} = \frac{V_{\text{red cells}}}{V_{\text{red cell}} + V_{\text{plasma}}} \times 100 \qquad (1)$$

The total body hematocrit (Htc_{body})

was determined from equation (1), the plasma and red cell volume in the body being determined from the amount of activity injected and the calculated initial concentrations of [51]Cr labelled erythrocytes and [131]I labelled albumin (GREGERSEN and RAWSON 1959).

The splanchnic hematocrit (Htc_{spl})

The splanchnic volume of plasma or red cells can be calculated from the equation

$$V_{\text{spl}} = \frac{\sum(c - c) \times t}{c} \times F_{\text{spl}} \qquad (2)$$

when the experimental figures for either red cells or plasma are employed.

By summing all the arterio-hepatic venous activity differences per unit of volume ($\sum(c - c)$) and multiplying with the duration of each sampling (t) and the splanchnic flow (F_{spl}), the amount of activity taken up in the splanchnic bed is obtained. Dividing this amount by the activity at equilibrium (c) in 1 ml of red cells or plasma respectively yields the splanchnic volume of red cells or plasma.

The mean splanchnic circulation time (MCT)

by definition is volume divided by flow, therefore from equation (2)

$$\text{MCT} = \frac{\sum(c -) \times t}{c} \qquad (3)$$

The extra plasma pool

The difference between the large vessel hematocrit and total body hematocrit may be expressed as an amount of extra plasma in the body. This extra plasma can be quantitated as the difference between the plasma volume measured and that predicted from the red cell volume and the hematocrit in the large vessels.

$$\text{Extra plasma} = V_{\text{plasma}} - V_{\text{red cells}} \left(\frac{100}{Htc_L} - 1\right) \qquad (4)$$

Results

Fig. 1 shows the curves obtained in a typical experiment. It is seen that the labelled red cells reach the hepatic veins at an earlier time than the labelled plasma, and also that identical concentrations of labelled cells in the arterial and the hepatic venous blood is more rapidly obtained than equal concentrations of labelled plasma.

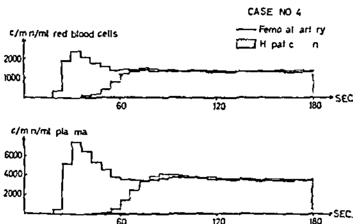


Fig. 1. Radioactivity in relation to time of labelled cells and plasma, simultaneous determinations in arterial and hepatic venous blood. The labelled cells and plasma were injected into an antecubital vein at 0 time.

This difference is expressed in the mean circulation times of plasma and red cells shown in Table I. MTC_{plasm} was on the average 44 sec varying from 23 to 58 and $MTC_{red\ cells}$ 37 sec varying from 20 to 46. No significant correlation was found between on one hand the mean circulation times and body weight, surface area, total volume of cells and the splanchnic flow on the other. Between MCT_{plasm} and the total plasma volume there was a small but statistically

Table II

Case no.	Large vessel hematocrit per cent	Body hematocrit per cent	Splanchnic hematocrit per cent	Body large vessel hematocrit ratio	Splanchnic/large vessel hematocrit ratio
1	32.8	27.4	29.9	.84	.91
2	36.1	33.1	31.0	[illegible]	.86
3	40.1	36.2	34.2	.90	.85
4	39.7	35.2	32.4	.89	.82
5	41.4	36.4	35.0	.88	.85
6	31.3	[illegible]	29.9	.88	.96
7	39.0	35.5	36.8	.91	.94
8	49.1	44.8	46.6	.91	.9
9	37.2	34.2	31	.9	.85
10	41.9	40.1	39.7	.96	.95
Average	38.9	[illegible]	34.7	.90	.89
S.D.	4.8	.2	5.2	.03	.06

S.D. = standard deviation.

Table III

Case no.	Body plasma volume ml	Body red cell volume, ml	Splanchnic plasma flow ml/min	Splanchnic plasma volume ml	Splanchnic red cell volume ml	Splanchnic extra plasma ml	Body extra plasma ml
1	2 980	1 130	1 050	400	170	0	660
2	2 440	1,210	560	420	190	80	300
3	2 830	1 610	930	300	470	200	490
4	2 930	1 600	720	650	310	180	500
5	3 220	1 850	1 130	920	500	10	600
6	3,270	1,240	1 070	470	200	30	5 0
7	2 920	1 610	650	550	320	50	400
8	2 780	2,270	1 170	790	680	90	430
9	2 770	1 440	500	430	190	110	340
10	4 020	2 700	580	420	270	0	280
Average	3 020	1 670	830	600	330	105	450
S D	475	500	260	200	170	0	130

S D = standard deviation.

significant correlation ($r = +0.65$, $p < 0.01$). The difference between the average splanchnic mean circulation time of plasma and that of red cells is not statistically significant but the average of the individual ratios is significantly different from unity ($p < 0.001$). On the average the mean circulation time of plasma in the splanchnic area is 20 per cent greater than that of the red cells. From this follows that the hematocrit value of the blood in the splanchnic vascular pool is smaller than that of the large vessels (Table II). The difference between the splanchnic hematocrit and large vessel hematocrit is statistically significant ($p < 0.001$) whereas the splanchnic and the total body hematocrit values are not significantly different ($p > 0.4$). On the average the splanchnic hematocrit was 89 per cent and the total body hematocrit 90 per cent of the large vessel hematocrit.

Table III states the splanchnic flow and volumes and the body volumes of plasma and red cells and the standard deviations. The splanchnic plasma flow given is the mean value of all determinations in each subject, *no systematic* variations in flow during the experiments were noted. In the last column the amount of extra plasma necessary to account for the difference between large vessel and total body and splanchnic hematocrit values are given.

Discussion

The ratio between body and large vessel hematocrit and its variation *from* subject to subject in the material presented is in agreement with the values

found by most other workers Furthermore the average hematocrit values of the body and the splanchnic circulation are almost identical as also found in a preliminary report by LATHEM and GORDON (1955) This shows that the difference between large vessel and body hematocrit is not exclusively caused by a low hematocrit value in the splanchnic area provided the determination of splanchnic and body hematocrit values are comparable

Usually the body hematocrit is determined from the concentrations of the plasma and red cell tracers 15 min after the injection with a standard correction for the elimination of ^{131}I albumin in this interval whereas the splanchnic hematocrit is determined during the first few minutes The body hematocrit may also be calculated from the same equilibration concentrations as used for determination of the splanchnic hematocrit without correction for elimination of albumin In our experiments the body hematocrit by the former method was 36 0 $\pm$ 5 2 and by the latter 35 0 $\pm$ 5 2 *i e* not statistically different.

Systematic errors may affect the measurement of the arterio hepatic venous difference It may be questioned whether a given sample is representative of all arterial or hepatic venous blood samples in the time interval during which is was drawn This question is in part answered by the close agreement between simultaneous determinations in two hepatic veins (TYGSTRUP WINKLER and ANDREE LARSEN 1961) but occasional errors of this kind cannot be excluded Determinations of body and splanchnic hematocrit by the method used in this and similar works depend on the assumption that labelled albumin and red cells injected intravenously are treated by the organism in the same way as albumin and red cells already present in the circulation

The most likely explanation of the difference between the large vessel and the body hematocrit is the presence of relatively more plasma than red cells in the peripheral circulation (including the rapidly exchangeable extravascular compartments) compared with the large vessels By the presented method of calculation the extra plasma of the body averaged 450 ml or 15 per cent of the plasma volume of the body

The close agreement between body and splanchnic hematocrit shows that the extra plasma of the body is not mainly found in the splanchnic area The average splanchnic extra plasma was found to 105 ml or 17 5 per cent of the splanchnic plasma volume The absolute figure is less accurate than the relative one because it depends on determination of the splanchnic plasma flow which is encumbered with a relative large experimental error

The amount of splanchnic extra plasma is of interest for two reasons Firstly the amount of extra plasma in the liver is of physiological significance especially with reference to the permeability of the hepatic sinusoids Determination of the splanchnic extra plasma does not however elucidate this point probably most of the splanchnic organs contain more extra plasma than apparent from the present figure as a high hematocrit assumably is found in the spleen quantitatively dependent on the mixing in that organ Secondly

if the hematocrit in some organs is different from the body hematocrit, the "extra" plasma of the body is not evenly distributed, and the contribution of each organ to a deviation of the body/large vessel hematocrit ratio from unity can only be evaluated from determinations of the "extra" plasma in each organ. The method therefore may contribute to localization and possibly to explanation of the body/large vessel hematocrit ratio if several parts of the circulation are investigated by this technique.

The findings in the present work does not contradict the theory that the low body hematocrit is caused by an excess of plasma in all of the small vessels in the body.

Appendix

The experimental data enter the general equations as follows:

[illegible equation] $F_{pl\ spl}$ (equation number illegible)

[illegible equation] $\frac{\mathrm{Hct}}{100-\mathrm{Hct}}$ (equation number illegible)

where

— [illegible] difference.

t [illegible] time after equilibration has taken place [illegible] represented by the time when continuous sampling was stopped.

c_e [illegible] equilibration, determined as the mean [illegible] in the last 10 samples [illegible] from the artery and [illegible] from the vein.

t [illegible] each sampling [illegible]

$F_{pl\ spl}$ [illegible] splenic [illegible] plasma flow [illegible]

Hct [illegible] vessel hematocrit.

H [illegible] = [illegible equation]

MCT [illegible] = [illegible equation]

MCT [illegible] = [illegible equation]

H [illegible] = [illegible equation] (10)

Extra plasma [illegible] = [illegible equation] $\frac{100-\mathrm{Hct}}{\mathrm{Hct}}$ (11)

Extra plasma [illegible] = [illegible equation] $\int$ [illegible] $F_{pl\ spl}$ (1?)

Table IV Estimated analytic l errors of splanchnic hematocrit mean circulation times volume and extra plasma (Av of 7 exp)

	M n	S D
Htc spl, per cent	36.2	± 0 6
MCT plasma, sec	4 2	± 0 8
MCT red cells, sec	36 0	± 0 6
Volum plasma ml	647	± 55
Vol m d ells, ml	400	± 33
Ext a pl sma ml	96	± 17

S.D = st ndard dev at o

Th analyt cal rro of the al es f tl planchnic y t m was estimated f r rr rs f — c and F_{pl} pL whil dis ga di g errors f t and Htc i

The err of — was d termined as th a t o f this alu n 10 pairs of samples dr wn f m 126 to 180 minutes ft the injecti n Th rr of was d t rmined as th ati n in u ity in 10 samples drawn 156 t 180 minutes, 5 arterial and 5 en us sampl s. These ariat ons incl de a poss bl b ological ar ation Three experim ts in h ch sampling f blood was topped between 120 and 180 minutes ar n t included Th m an tandard devi t ons f th emaining 7 experiment w calculat d Th rro of F_{pl} pL as calculat d as th riat on f th 6 d t rmin t n perfo med in each ubj t

F m th m an tanda d d v t ons th ar t n of th data tering th quati ns ere calculated. In th case of Σ(a—c)pL t am unts to 1 9 pe ent f th erag alue measured f pl to 0 3 per c nt of (a—c e to 1 5 pe c nt and f t 0 5 per cent Th mean error of $F_{pl,spl}$ is 8 2 pe ent

The influ nce of these rr rs in th final calculati ns was estimated f m th coeffic ents of ariati (HALD 195) Th rr f the Ht pL and MCT is bout 2 per cent, that of th splanch nic olumes about 8 pe cent, and that f th ext plasma pL about 18 pe cent From these variat oeffici nt nd th m an al es of th experim nts ntering thi calc lati n th absol t tandard d iat ons assessed (Tabl IV)

The th rs wish to thank Mrs B M LLER PETERSE and Miss L HA SEN Department of Clinical Chemistry Bisp bj g H p tal fo in luabl techn cal assistan

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Der Einfluss anhaltender hoher O Konzentration der Einatmungsluft (30—100 %) auf die Erythropoiese der Ratte

on

BERNHARD TRIBUKAIT

Eingeg ngen am 11 September 1962

Abstract

TRIBUKAIT B *Der Einfluss anhalt nder hoher O K n ent ation der E natmung luft (0—100) auf die Erythropoiese de Ratte* A ta phys ol. cand. 1963 57 407—418 — Rats were subj ted to oxygen con centrations of 30—100 fo 8—10 weeks Total hemoglobin, blood volume and relat e blood values were determined The lungs were unaffected by oxygen concentrations up to 80 The total hemoglobin and the blood values emained essent ally normal although the reticulocytes occasionally decreased pp iably Acute and chronic lung les ons developed in rats subj cted to 100 oxygen concentration. If they surv ed the nitial lesions they were left in the 100 oxygen conc ntration for 10 w ks and wer then placed in an environment of oom air for 6 weeks Upon return to 100 oxygen concentration no further acute lesions of the lungs occurred The erythropoiesis was markedly influenced by the lung damage

Wahrend für niedrigen Sauerstoffdruck der Einatmungsluft eine stimulie rende Wirkung auf die Erythropoiese gesichert ist sind die Resultate des Einflusses hohen Sauerstoffdrucks wesentlich unklarer Eine Übersicht der Literatur zu dieser Frage findet sich bei BEAN (1945) sowie GRANT und ROOT (1952) Vielfach sind leicht erniedrigte relative Blutwerte gefunden worden (BORNSTEIN 1911 CAMPBELL 1927 a 1927 b BOYCOTT und OAKLEY 1933 COOPERBERG und SINGER 1951 GYLLENSTEN und SWANBECK 1954) Es liegen aber auch gegensatzliche Befunde vor (ACHARD BINET und LEBLANC 1927 PAINE, KEYS und LYNN 1941 Neben der Hohe des Sauerstoffdrucks und

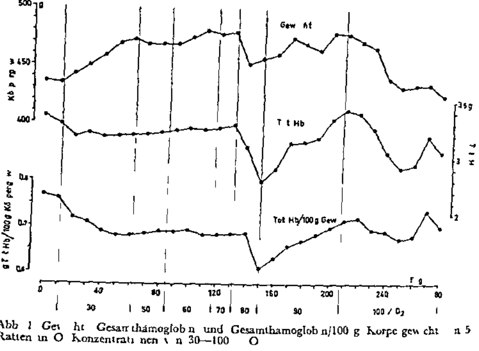


Abb 1 Gewicht Gesamthämoglobin und Gesamthämoglobin/100 g Körpergewicht von 5 Ratten in O_2 Konzentrationen von 30—100 % O_2

Tierart spielen dabei zeitliche Faktoren eine gewisse Rolle Nach einsetzendem hohen Sauerstoffdruck kommt es zu offenbar vorübergehenden Plasma volumenveränderungen (ANTHONY 1939 1940 BINET und BOCHET 1941 LAMARCHE und ARNOULD 1957) Im weiteren Verlauf können Lungenschäden als typische Komplikation das Bild weitgehend beherrschen (BECKER FREYSENG und CLAMANN 1939 CLAMANN *et al* 1940 PAINE *et al* 1941)

Die vorliegende Arbeit stellt die Fortsetzung von Studien über den Einfluss unterschiedlichen Sauerstoffdrucks auf die Erythropoiese dar Dabei war zunächst nur die Wirkung von Hypoxie untersucht worden (TRIBUKAIT 1963 a) Um mit grösserer Sicherheit die Erythropoiese quantitativ beurteilen zu können erscheinen auch hier Gesamt Hb Bestimmungen unumgänglich Solche haben offenbar nur BOYCOTT und OAKLEY (19‿3) unter diesen Bedingungen mit der WELCKER schen Extraktionsmethode durchgeführt

Methodik

Die Versuche wurden an männlichen Wistar und Kragenratten (Hooded rats, National Institute for Medical Research Mill Hill London) vorgenommen Gruppen von 4—5 Tieren wurden in etwa 30 l grossen Glasbehältern die luftdicht zu verschliessen waren, bei Zimmertemperatur gehalten Die Behälter wurden durch eine Pumpe mit einem O_2 Luftgemisch bzw reinem O_2 mit 4 l/Min durchlüftet D *Gemisch* konzentration wurde mit Hilfe guter Nadelventile unter Kontrolle eines *Beckman O* Analysators und Gasanalysen nach HALDANE eingestellt und täglich überprüft *Die*

Tab I Gewicht und Gesamthämoglobin von 5 Ratten in erhohter O_2 Konzentration zwischen 30 und 100 Durchschnittswerte der verschiedenen Versuchsabschnitte

		Versuchs dauer (Tage)	Gewicht (g)	Total Hb (g)	g Tot. Hb / 100 g Gew
Luft			436 ± 12 8	3 34 ± 0 083	0 77 ± 0 006
30	O	51	458 ± 10 0	3 15 ± 0 057	0 69 ± 0 008
50	O	23	4 0 ± 9 7	3 16 ± 0 085	0 67 ± 0 015
60– 0	O	46	4 8 ± 11 1	3.27 ± 0 050	0 68 ± 0 017
80–90	O	6	467 ± 12 4	3 08 ± 0 114	0 66 ± 0 010
100	O	72	448 ± 9 2	3 18 ± 0.224	0 71 ± 0 037

CO_2-Konzentration in den Kammern uberstieg nicht 0 5 D e T e e erhielten ein spezielles Rattenbrot (Zusammensetzung s TRIBUKAIT 1960 a) sowie Hafer Mohr ruben und Wasser *ad l bitum* Zum Reinigen der Behalter wurden die T ere alle 2—3 Tage fur etwa 10 Min. Normalluft ausgesetzt, ebenfalls fur die Untersuchungen die 1 1/2 Stunden nicht uberschritten

D e Gesamt Hb-Menge wurde mit der sogenannten alveolaren CO Methode bestimmt, mit der Messungen am selben T er beliebig oft wiederholt werden konnen (TRIBUKAIT 1960 b) Das Blut Plasma und Erythrozyten olumen wurden aus dem Gesamt Hb und aus der Hb-Konzentration bzw dem Hamatokrit unter Berucksichtigung des Korperhamatokrit n der fruher angegebenen Weise berechnet (TRIBUKAIT 1960 b) D e Ret culo yten von 2 × 1 000 Erythrozyten urden entsprechend der Methode von LARSSON und SWENSSON (1949) (Fixieren dunner Blutausstriche in 1 iger Sublimatlosung Farben mit Toluidinblaulosung 0 75 pH 5 7) bestimmt D e Erythrozyten wurden in einer Burkerkammer ausgezahlt

Ergebnisse

In einer ersten Versuchsserie sollten allgemeine Erfahrungen uber die Vertraglichkeit verschiedener O Konzentration und gleichzeitig das Verhalten des Gesamt Hb gesammelt werden Dabei wurden 8 Tiere (Wistar) von denen 5 die Versuchszeit von uber 9 Monaten uberlebten schrittweise erhohter O Konzentration bis zu reinem O_2 ausgesetzt Das Ausgangsgewicht mit durchschnittlich 435 g war mit Absicht hoch gewahlt worden um weniger abhangig vom Gewichtszuwachs zu sein der die Beurteilung der Veranderungen des Gesamt Hb erschweren kann Das Gesamt Hb wurde in etwa 5 tagigen Zeitintervallen gemessen

Die Resultate dieses Versuchs zeigt Abb 1 jeweils 2 Messwerte sind in einem Punkt zusammengefasst In Tab I sind ferner die Mittel aller Werte die unter den verschiedenen O Konzentrationen gefunden worden sind aufgefuhrt Das Gesamt Hb mit 3 3 g bei Versuchsbeginn ist etwas hoher als der Durchschnitt gleichgrosser Tiere dieses Stammes (TRIBUKAIT 1960 a) weicht aber nicht signifikant davon ab

Tab. II. Gewicht, Gesamthämoglobin, Blutvolumen und relative Blutwerte von 8 Ratten nach durchschnittlich 67 tägigem Aufenthalt in 100 % O_2 und nach einem darauffolgendem 45 tägigem Aufenthalt in Luft

	Gewicht (g)	Tot. Hb (g)	g Tot. Hb/100 g Gew.	Blutvol. (ml)	Hb conc. (g%)	Hct (%)	Ery mill./mm³
100 % O_2	394 ± 15.5	2.98 ± 0.120	0.75 ± 0.021	31.5 ± 2.41	12.31 ± 0.287	43.2 ± 1.16	7.0 ± 0.36
Luft	423 ± 13.0	3.17 ± 0.098	0.75 ± 0.010	29.3 ± 1.23	15.39 ± 0.064	53.0 ± 0.90	9.4 ± 0.22

Während der 50 tägigen Periode in 30 % O_2 stieg das Körpergewicht um etwa 10 %; bei gelegentlich durchgeführten Obduktionen solcher Tiere fiel ein ungewöhnlich hoher Fettgehalt auf, der sonst nicht beobachtet worden ist. Das Gesamt-Hb fiel gleichzeitig etwas, die Differenz ist aber nicht signifikant. Der Abfall des Gesamt-Hb/100 g Körpergewicht von 0.77 g auf 0.69 g ist zwar hochsignifikant ($p < 0.001$), das Gesamt-Hb entspricht aber jetzt ganz dem Normalwert dieser Tiergrösse.

Während der nachfolgenden 70 Tage bis zu einer O_2-Konzentration von einschliesslich 70 % sind alle Werte praktisch unverändert. Die Tiere befanden sich in sehr gutem Allgemeinzustand.

Beim Übergang auf 80 % erkrankten die Tiere an einer Luftwegsinfektion, z. T. verbunden mit schwerer Dyspnoe. Dabei fielen Gewicht und Gesamt-Hb hochsignifikant ($p < 0.001$) ab. Die Tiere erholten sich jedoch relativ rasch und erreichten, obwohl die O_2-Konzentration auf 90 % erhöht wurde, wieder ihr Höchstgewicht und Gesamt-Hb-Werte wie zuvor.

Reinen O_2 (ca. 99 %) vertrugen die Tiere nur etwa 20 Tage ohne sichtbare Schäden oder Veränderungen in ihrem Verhalten. Danach sank definitiv das Körpergewicht, ebenso das Gesamt-Hb. 2 der Tiere, die stark dyspnoisch geworden waren, wurden schliesslich getötet. Die Obduktion ergab multiple Lungenabszesse und pleuritische Veränderungen. Die 3 anderen Tiere waren jedoch unauffällig.

Nach den Ergebnissen der ersten Versuchsserie schien es möglich, wenn auch nur in begrenztem Umfang, Tiere für längere Zeit in relativ gutem Zustand in reinem O_2 zu halten. Es besteht jedoch die Möglichkeit, dass die Erythropoiese durch Folgen der durchgemachten Lungenerkrankungen (O_2-Diffusionsstörungen) stärker beeinflusst werden kann. In einem solchen Fall sind nach Übergang auf Luftatmung Veränderungen der Erythropoiese wie sie bei Hypoxie auftreten, denkbar.

In wie weit das zutrifft, sollte durch die zweite Versuchsserie geklärt werden. Zu diesem Zweck wurden Tiere (Wistar) direkt reinem O_2 ausgesetzt. Regelmässig traten dabei nach 24—48 Stunden Lungenerkrankungen mit starker Dyspnoe auf. Etwa 60 % der Tiere starben. Die Lungen dieser Tiere hatten

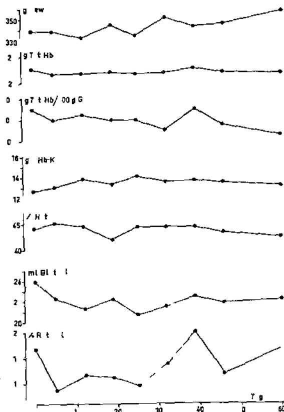


Abb 2 Gewi ht Gesamthamo globin Bl t lumen und lat B twe t on 10 Ratten in 80 O Dem Ausgangswert (Tag 0) 1 eg n D ppelbestimmungen be Luftat mung i grund

leberartiges Aussehen und leberartige Konsistenz in den Pleuren fanden sich vielfach mehrere ml einer blutig serosen Flussigkeit Die anderen Tiere erholten sich jedoch und zeigten im weiteren Verlauf kein abnormes Verhalten

8 derartige Tiere befanden sich zwischen 48 und 78 Tagen in reinem O Da die Rekonvaleszenz unterschiedlich lang war und aus ausseren Grunden der Zeitpunkt des Versuchsbeginns differierte wird hier auf eine graphische Darstellung der gefundenen Gesamt Hb Werte und des Korpergewichts verzichtet In Tab II sind die Mittel der Gewicht und Blutwerte nach durch schnittlich 67 tagigem O_2 Aufenthalt und nach einer nachfolgenden Periode von 45 Tagen in Luft zusammengestellt In beiden Fallen hatten sich relativ konstante Gewichts und Gesamt Hb Werte eingestellt Wahrend die Absolut blutwerte keine statistisch zu sichernde Differenzen zeigen unterscheiden sich die relativen Blutwerte hochsignifikant ($p < 0\,001$) voneinander Daruber hinaus weichen sie auch vom Mittelwert eines grosseren Normalmaterials (TRIBUKAIT 1960 a) signifikant ($p \cong 0\,01$) ab im einem Fall in Richtung auf eine Anamie im anderen auf eine Polyzythamie

Tab III Geuicht Gesamthamoglobin Blutvolumen und relatiue Blutuerte on 10 Ratten vor (I)

	Gewicht (g)	Tot Hb (g)	g Tot Hb/ 100 g Gew	Rel Hb (g)	Hct ( )
I	339 ± 18 6	2 26 ± 0 100	0 67 ± 0 013	12 64 ± 0 199	44 1 ± 0 54
II	3 8 ± 20 4	2 17 ± 0 0 6	0 62 ± 0 022	13 10 ± 0 175	42 4 ± 0 47
D ff II—I	18 6 ± 8 28	−0 09 ± 0 061	−0 054 ± 0 0173	0 463 ± 0 3083	−1 70 ± 0 730
t	2 246	1 474	3 121	1 502	2 329
p	0 05 0 01	> 0 05	0 01—0 001	~ 0 05	0 05—0 01

Der Versuch wurde damit fortgesetzt dass die Tiere erneut reinem O_2 ausgesetzt wurden Dabei traten keinerlei Zeichen einer erneuten Lungen erkrankung auf Das Gewicht fiel jedoch wiederum auf 390 g das Gesamt Hb auf 2 8 g Der Versuch fand durch Versagen der Pumpe nach 38 Tagen ein Ende Makroskopische Lungenveranderungen fanden sich nicht mikroskopische Untersuchungen liegen nicht vor Auffallig war das mit einem Durch schnittsgewicht von 1 7 g um etwa 75 % uber dem Normalwert liegende Herzgewicht das auf die Moglichkeit auch anderer Organveranderungen unter Hyperoxie hinweist

Die Resultate der zweiten Versuchsserie ermoglichen zwar ni ht im Ein zelnen die Veranderungen der Erythropoiese durch eine direkte O Einwirkung von denen einer indirekten durch sekundare Organveranderungen abzugrenzen Letztere erschienen jedoch so nahe dem Bereich des Moglichen zu liegen dass detailliertere Untersuchungen der Erythropoiese nur in 80 % O vorgenommen wurden obgleich prinzipiell auch in 100 % O_2 ein langerer mit dem Leben zu vereinender Aufenthalt moglich ist

In der dritten Versuchsserie wurden 10 Kragenratten fur 60 Tage direkt 80 % O_2 ausgesetzt Wahrend der gesamten Versuchszeit waren keinerlei Anzeichen irgendwelcher Gesundheitsstorungen speziell seitens der Respira tionsorgane (Dyspnoe Husten) zu beobachten die sonst nicht selten auch bei Tieren unter Normalbedingungen auftreten konnen Die Resultate zeigen Abb 2 sowie Tab III der auch die Differenzen zwischen Ausgangswerten und Endwerten zu entnehmen sind Bei dem sehr guten und homogenen Tierma terial findet sich zwar vielfach eine Signifikanz der Differenzen die Unter schiede sind jedoch absolut gesehen ausserst bescheiden

Bei einem Gewichtsanstieg um 10 % ist das Gesamt Hb unverandert Damit fallt das auf das Korpergewicht bezogene Gesamt Hb (g Hb/100 g Korpergewicht) von 0 67 g auf 0 62 g Fur Blut und Plasmavolumen gilt entsprechendes Bei konstanter Hb Konzentration sinkt der Hamatokrit etwas d h die mittlere Erythrozyten Hb Konzentration (Hb-Konz × 100/Hct) steigt von 28 7 auf 30 9 Die markantesten Veranderungen erfahren die Reti

und na h 60 tag gem Aufenthalt in 80 O (II) mit D fferen und S gnifikan der D fferen

Rel. Hb. (g) / Hct. ( ) 100	Blut l (ml)	ml Blut vol / 100 g Gew	Ery ol (ml	Plasmav l (ml)	R t (/)
287 ± 0 21	23 9 ± 0.99	7 1 ± 0 14	7 9 ± 0 32	16 0 ± 0 68	16 7 ± 2.03
30 9 ± 0 39	22.1 ± 0 91	6 ± 0.21	7 0 ± 0 31	15 1 ± 0 61	16 9 ± 2.17
2 25 ± 0 425	−1 72 ± 0 355	−0 84 ± 0.214	−0 85 ± 0 149	−0 87 ± 0.291	0 19 ± 2 361
5.294	4 845	3 916	5 716	2 985	0 080
< 0 001	< 0 001	0 001	< 0 001	0 01 – 0 001	> 0 05

culozyten die innerhalb von 5 Tagen von 16 % auf 8 / signifikant (p = 0 01) abfallen auf diesem niedrigen Niveau etwa 25 Tagen bleiben und dann wieder auf den Ausgangswert ansteigen

Diskussion

Anhaltend hohe O Konzentration der Einatmungsluft hat im Gegensatz zu niedriger Konzentration nach den vorliegenden Untersuchungen eine unbedeu tende oder keine Wirkung auf Gesamt Hb und relative Blutwerte In einer kritischen Beurteilung der derzeit vorliegenden Untersunchugen sind GRANT und ROOT (1952) zu einem ahnlichen Schluss gekommen Im Gegensatz zu diesem geringen Effekt *in vi o* scheint die praktisch vollstandige Unterdruckung der Erythrozytenbildung von Knochenmark *in vitro* zu stehen wenn die O_2 Konzentration etwa 50 uberschreitet (MAGNUSSEN 1949) Die verminderte Aufnahme der Erythrozyten von Fe bei 85—95 iger O_2 Atmung haben JACOBSON und Mitarbeiter (1957 a b 1959) ebenfalls im Sinne einer gehemm ten Erythropoiese gedeutet

Fur eine Diskussion dieser Befunde ist zunachst notwendig sich uber die in diesem Zusammenhang wesentlichen Veranderungen klar zu werden die die Atmung hoher O_2 Konzentration mit sich bringt Die markantesten Ver änderungen erfahrt der arterielle O Druck der mit etwa 100 mm Hg bei Luftatmung und etwa 640 mm Hg bei reiner O Atmung das Mehrfache des Normalwertes erreichen kann Demgegenuber steigt der arterielle O Gehalt nur mit der physikalisch gelosten O Menge d h um rund 0 0031 ml O /ml Blut/100 mm Hg O sowie durch die etwas hohere O_2Hb-Sattigung

Schwieriger sind die Veranderungen auf dem Gewebsniveau zu uberblicken Dabei interessiert besonders der O Druck des Gewebes Dieser hangt vom O_2-Verbrauch des Gewebes den O Diffusionsverhaltnissen (Kapillarisation Diffusionskoeffizienten O Druckdifferenzen) und der Zirkulation im Kapil larsystem ab THEWS (1960) hat kurzlich die Bedeutung verschiedener Kom ponenten dieses komplexen Zusammenhangs am Beispiel der O Diffusion i

Gehirn ausfuhrlicher quantitativ behandelt Hier sein nur einige allgemeine qualitative Überlegungen angestellt

Beim Übergang von Luft auf O_2 Atmung wird zunachst der Stickstoff des Gewebes (~ 570 mm Hg = arterieller N_2 Druck) an das Blut bzw die Ausatmungsluft abgegeben Verbrauchte das Gewebe keinen O_2 erreichte der O_2 Druck an Stelle dessen den gleichen Wert wie im arteriellen Blut Da aber im Gewebe O_2 verbraucht wird hangt der O_2 Gewebsdruck neben den Diffusionsverhaltnissen im wesentlichen vom O_2 Verbrauch und der kapillaren Durchblutung ab Ein Absinken der Pulsfrequenz mit einem Abfall des Herzminutenvolumens unter O_2 Atmung scheint fur den Menschen gesichert zu sein (Literatur s STORSTEIN 1952 COMROE und DRIPPS 1953 DALY und BONDURANT 1962) Gleichzeitig scheint der O_2 Gehalt des gemischten venosen Blutes und auch der O_2 Druck der dem O_2 Gewebsdruck am nachsten kommt trotz der hoheren physikalisch gelosten O_2 Menge des arteriellen Blutes gegenuber Luftatmung nicht oder kaum verandert zu sein (STORSTEIN 1952, GRANATH 1962) Damit wird wahrscheinlich dass unter O_2 Atmung der O_2 Gewebsdruck mit Ausnahme der den arteriellen Kapillarabschnitten am nachsten gelegenen Gewebszellen praktisch unverandert ist Berechnungen des O Gewebsdrucks aus dem O Druck intraperitoneal oder subcutan injizierten Stickstoffs stutzen diese Überlegungen im wesentlichen TAYLOR (1949) sowie BAHNSON und MATTHEWS (1953) fanden praktisch normale O_2 Druckwerte unter der Voraussetzung dass keine Lungenschaden vorlagen CAMPBELL (1927 a) bei 2 Kaninchen normale subcutane und leicht erhohte abdominale O Druckwerte

Relatiert man die Befunde von Gesamt und Relativ Hb unter O Atmung verschiedener Konzentration zu dem damit veranderten O_2 Druck und Gehalt von Blut und Gewebe ergibt sich folgendes Bild

1 Hoher *arterieller O Druck* scheint keinen wesentlichen Einfluss auf die Erythropoiese zu haben Auch unter Hypoxie scheint der erniedrigte arterielle O Druck nicht unmittelbarer Anlass der erhohten Erythropoiese zu sein Die strenge Zuordnung der gesteigerten Erythropoiese unter Hypoxie zur absinkenden O Hb Sattigung des arteriellen Blutes spricht eindeutig mehr fur die uberwiegende Bedeutung des arteriellen O_2 Gehalts als des arteriellen O_2 Druckes (TRIBUKAIT 1962 a)

2 Der *arterielle O Gehalt* steigt bei reiner O_2 Atmung um eine O Menge die der Bindung kapazitat von etwa 2 g % Hb enspricht Diese Steigerung leitet sich teils aus der hoheren physikalisch gelosten O_2 Menge teils aus der Vollsattigung des Hamoglobins mit O her das sich von der O_2Hb Sattigung bei Luftatmung um einige Sattigungsprozente unterscheidet Es ist prinzipiell denkbar dass die Hb Konzentration bei O Atmung um einen derartigen Betrag abfallt Die relativ niedrige Hb Konzentration von *12 3 g % in den* vorliegenden Versuchen kann zwar eventuell in diesem Sinne gedeutet werden Es durfen aber nicht zufallige Plasmavolumenvariationen oder mit O_2 Atmung in Verbindung stehende Flussigkeitsverschiebungen (LAMARCHE und ARNOLD

1957) und vor allem nicht pathologische Organveranderungen als Ursache ausser Acht gelassen werden Die Versuche in 80 % O mit einer vollig unveranderter Hb-Konzentration sprechen fur letztgenannte Moglichkeit Im ganzen gesehen sind die O_2 Menge um die es geht so gering und sekundare Komplikationen derartig naheliegend dass etwaige niedrige Hb-Konzentrations-Werte bei O_2 Atmung im allgemeinen nur mit Zuruckhaltung im Sinne eines kompensatorischen Abfalls aufzufassen sein durften

3 *Dem O Gewebsdruck* kommt besonderes Interesse zu da eine direkte Abhangigkeit regulatorischer Vorgange der Erythropoiese von diesem vermutet werden konnen Wie oben ausgefuhrt ist zumindest zweifelhaft ob uberhaupt der O Gewebsdruck unter O Atmung ansteigt Die im wesentlichen unveranderten Gesamt und Relativ Blutwerte des Versuchs bei 80 / iger O_2 Atmung wobei keinerlei Zeichen von Lungenschaden festzustellen waren konnen in diesem Sinne gedeutet werden

Beurteilte man die Erythropoiese nur nach der Reticuloztenzahl konnte man allerdings leicht zu einer anderen Auffassung gelangen Bedeutete der gefundene Abfall der Reticulozyten um die Halfte eine Einschrankung der Hb-Bildung um 50 / sollte bei einer fur diesen Tierstamm gefundenen Erythrozytenlebenszeit von rund 55 Tagen (FORSSBERG und TRIBUKAIT 1962) das Gesamt Hb nach 10 Tagen von 2 26 g um rund 10 / auf etwa 2 g nach 20 Tagen um etwa 0 4 g u s w gesunken sein Das ist aber nicht der Fall Rein theoretisch betrachtet lasst sich zwar nicht die Moglichkeit ausschliessen dass hier der Zellabbau gehemmt wird und damit verbunden auch die Zellneubildung Das bedeutet jedoch praktisch dass unter Normalbedingungen gebildete Erythrozyten deren aussere Lebensgrenze bereits zum Zeitpunkt ihrer Bildung im wesentlichen bestimmt sein durfte unter den herrschenden Bedingungen ihre mittlere Lebenslange plotzlich wesentlich steigern Eine sichere Basis fur die Annahme einer derartigen drastischen Anderung im Ablauf der Alterungsprozesse dieser Zellen lasst sich vorlaufig nicht erkennen zumal da eine eindeutig verlangerte Lebenszeit unter verschiedensten Bedingungen gebildeter Erythrozyten bisher nicht zu finden gewesen ist (BERLIN WALDMANN und WEISSMAN 1959)

Mit der Frage in wie weit die Reticulozyten den Funktionszustand der Erythropoiese widerspiegeln wird ein wichtiger Punkt beruhrt der generell alle entsprechenden Untersuchungen der Erythropoiese betrifft Selbstverstandlich konnen veranderte Reticulozytenwerte echte Variationen der Erythropoiese anzeigen Das Knochenmark dient aber nun nicht nur als Bildungsstatte sondern auch als Depot der Reticulozyten und kann auf verschiedene Reize empfindlich mit Durchblutungsanderungen reagieren (CLEMMING 1962) Vor allem aus kurzfristigen Reticulozytenveranderungen auf spezifische erythropoietische Veranderungen zu schliessen kann irrefuhrend sein Eine Reticulozytose kann z B nach Rontgenbestrahlungen (TRIBUKAIT nicht veroffentlicht) nach Reizung von Hirnpartien (Luftfullung der Hirn

ventrikel, Angiographie, Hypoxie u. a. — BEER 1942, SEIP 1953, BOE und BENESTAD 1954) auftreten, ohne dass sich daraus irgendwelche Folgerungen für die Regulation der Erythropoiese herleiten liessen.

Aber selbst langfristige erhöhte Reticulozytenwerte im Blut müssen kritisch beurteilt werden. So erfolgt z. B. nach vielfach wiederholter Injektion von Co eine anhaltende Reticulozytose, ohne dass zunächst die Gesamt-Hb-Menge ansteige oder eine Hämolyse festzustellen wäre (TRIBUKAIT 1962 b).

Die erhaltene niedrige Reticulozytenzahl bei Atmung hoher O_2-Konzentration kann entsprechend nicht als sicheres Zeichen einer gehemmten Erythropoiese angesehen werden. Damit erscheinen *niedrige Reticulozytenwerte*, die REINHARD *et al.* (1944) sowie TINSLEY *et al.* (1949) bei Patienten mit Anämien verschiedener Genese unter O_2-Atmung beschrieben und als Ausdruck einer gehemmten Zellneubildung gewertet haben, in einem etwas anderen Licht. Bei einem Teil der Patienten traten ausserdem Komplikationen auch seitens der Respirationsorgane auf, die die Beurteilung zusätzlich erschweren. FRIED *et al.* (1957) haben die bei 85—95 %iger O_2-Atmung gehemmte Fe^{59}-Erythrozyten-*Inkorporation* bei Ratten — eine Art zeitliche Integration der Reticulozytenwerte — ebenfalls als Beweis einer gehemmten Erythropoiese gewertet. Da die Versuche nach 7—10 Tagen in O_2 vorgenommen wurden, die Tiere aber nicht mehr als 14 Tage O_2-Atmung überlebten, sind auch hier zusätzliche Komplikationsmöglichkeiten durch pathologische Veränderungen diskutabel. Der von LINKE (1962) beschriebene fehlende Abfall der Reticulozyten nach Ausschalten gewisser Hirnzentren und O_2-Atmung kann ebenfalls nicht als eindeutiger Beweis eines zentralnervösen Regulationsmechanismus der Erythropoiese angesehen werden.

Die Befunde von BOYCOTT und OAKLEY (1932), dass Ratten auch längere Zeit in reinem O_2 leben können, bestätigen die vorliegenden Untersuchungen. Dabei scheinen die Tiere einen gewissen Grad von langer anhaltender Akklimatisation zu erlangen. Tiere, die 9 Wochen *in reinem O_2* gehalten waren und nach 6 Wochen Aufenthalt in Normalluft wieder reinem O_2 ausgesetzt wurden, *erkrankten in keinem Fall* erneut an einer akuten Lungenveränderung. Demgegenüber waren bei dem ersten O_2-Aufenthalt alle Tiere erkrankt. Eine gewisse Akklimatisation kann offenbar auch durch intermittierenden hohen O_2-Druck erreicht werden (TAYLOR 1949). Da aber pathologische Lungen- und Kreislaufveränderungen unter diesen Bedingungen nicht ausgeschlossen werden können, sind weitgehendere Folgerungen aus Untersuchungen der Erythropoiese nur mit Reservation möglich.

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Die Readaptation der hohenpolyzythämischen Ratte an Meereshöhe

v n

BERNHARD TRIBUKAIT

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Abstract

TRIBUKAIT B *D e Readaptation der hohe p lyzythamischen Ratt an Meereshohe* Acta physiol scand 1963 *57* 419—430 — Rats were submitted to hypoxia corresponding to 6 000 m altitude f r 40—50 days. The total amount of hemoglobin, blood volume and relative blood values had been studied in hypoxia and after transfer to normal oxygen pressur Total hemoglob n increased during hypoxia by 150—200 lati e blood values by 100 / and blood volume by 25—50 / indicating the ncrease of oxygen capa ty of the blood and the volum of the circulatory system 30 days after transfer to normal oxygen p essure total hemoglob n blood olume and relative blood alues had returned to normal. Thereafter relati e blood values ho ed a trans ent d crease but not the total hemoglobin Thus th blood volume increased again. Problems in connection th the homoistatic gulation of erythropoiesis ha been discussed The body weight decreased during hypoxia by about 15 After the period of hypoxia as over th rat of increase in body eight was faster than that of animals of the same ord and body weight

Die beim Sauger unter chronischer Hypoxie auftretende sekundare Polyzythamie ist von absoluter Natur d h die Hamoglobinkonzentration des Blutes steigt als Folge einer vergrosserten Gesamthamoglobinmenge. Nur während der ersten Tage nach einsetzender Hypoxie steigt die Hb Konzentration als Folge eines Plasmaverlusts Daruber hinaus erfährt aber auch das Blutvolumen einen Anstieg

Diese Veranderungen die bei der Ratte im Einzelnen untersucht worden sind (TRIBUKAIT 1962 a b) beziehen sich also auf zwei verschiedene C

teils auf die Sauerstoffkapazitat des Blutes teils auf das Volumen des Gefasssystems fur welches das Blutvolumen ein Mass bildet Die Sauerstoffkapazitat steigt dabei bis zu einem Hypoxiegrad entsprechend etwa 6 000 m Hohe in ungefahr gleichem Umfang wie die arterielle O_2Hb Sattigung sinkt wodurch *der arterielle Sauerstoffgehalt bis zu dieser Hohe auf etwa normalem Niveau* gehalten wird (TRIBUKAIT 1962 c) *Die Vergrosserung des Gefässystems betrifft* in gleicher Weise das Herz (TRIBUKAIT 1962 e) die grosseren Gefasse (ANTHONY und KREIDER 1961) und die Kapillaren (OPITZ und SCHNEIDER 1950) Die erhohte Kapillarisation stellt dabei einen fur die Sauerstoffversorgung des Gewebes wichtigen Anpassungsprozess dar deren funktionelle Bedeutung von MERCKER und OPITZ (1949) noch uber den Anstieg der Sauerstoffkapazität des *Blutes oder eine Durchblutungszunahme gestellt* wird *Das anatomische* Bild *der Kapillaren entspricht dabei dem einer strukturell fixierten* Hypertrophie (OPITZ 1952 — Retinagefasse)

Es ist bekannt dass sich beim Übergang von O_2 Mangelbedingungen zu normalen O Druck Verhaltnissen auch die sekundare Polyzythamie zuruck bildet Entsprechend dem zwiefaltigen Charakter der Anpassungsprozesse an Hypoxie — der erhohten O Kapazitat des Blutes und der erhohten Kapillarisa*tion mit dem Anwachs des Gefassystems* die beide die O_2 Versorgung des Gewebes begunstigen — hat auch die Readaptation an normale O_2 Druck Verhaltnisse zwei Seiten Hinsichtlich der Ruckbildung der erhohten O_2 Kapazitat sind aktive hamolytische Prozesse und die Erythropoiese hemmende Einflusse diskutiert worden Die Ruckbildung des Gefassystems steht zwar im Zusammenhang mit der Normalisierung der O Kapazitat ubersteigt *aber den Rahmen eines qualitativen hamatologischen Problems Es stellen* sich hier Fragen *nach einer optimalen und adaquaten* O_2 Versorgung des Gewebes — einem Problemkomplex, der zwar unmittelbar das Regulationsziel der Erythropoiese betrifft bei dem die Erythropoiese jedoch nur ein Faktor unter mehreren darstellt

In der vorliegenden Arbeit sind die Ruckadaptationsprozesse beim *Übergang von Hohe auf Meereshohe verfolgt worden* Neben *den relativen* Blutwerten *wurden* um *quantitativ die Erythropoiese erfassen* zu konnen und um ausser dem eine Auffassung von den Veranderungen des Gefassystems zu erlangen auch Gesamthamoglobin und Blutvolumen untersucht Derartige Studien scheinen in nur sehr beschranktem Umfang vorzuliegen

Material und Methodik

Die Untersuchungen wurden an mannlichen Ratten eines Stammes (Wistar) vorgenommen Das Durchschnittsgewicht von zwei Versuchsgruppen betrug bei Versuchs beginn rund *290 g das einer weiteren Gruppe rund 350 g* Die Tiere wurden in Unterdruckkammern Hypoxie entsprechend 6 000 m Hohe 43 bzw 48 Tage lang ausgesetzt. Als Futter diente ein spezielles Rattenbrot (Zusammensetzung s TRIBUKAIT 1960 a) sowie Hafer Mohrruben und Wasser *ad libitum*

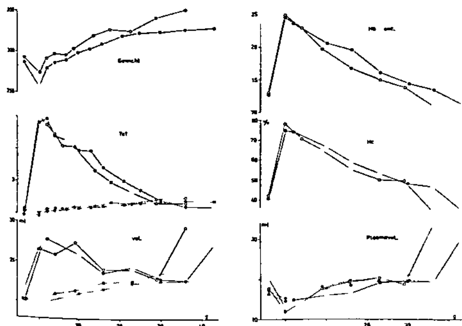


Abb. 1 Gew cht und Blutwe te on R tt n in M esh h n h 43 tägig m Auf nthalt in Hypoxi entsp e hend 6 000 m Hoh und n ch Übe gang uf M eshohe (T g 0) M tt l w t n 2 T ergruppen on j 6 (off Symbol) bzw 8 (ges hl ss ne Symbol) T e n Die Normalwe t f G mth m gl b Bl t und Plasm lumen fur T e e ntsp ech nde G sse md gestrich lt inge e hn t

D e Gesamthamoglob nmenge wurde mit e ner modifi rten alveolaren CO Methode (TRIBUKAIT 1960 b) unter Berucksichtigung der spezi llen Atmungsverhaltnisse be m hohen dapt erten T (TRIBUKAIT 1962 a) bestimmt Dabei befindet sich das Tier in ei em geschlossenen At msystem dem et as CO zugefuhrt ird solange bis sich e n Gleichgew cht zwischen Atemgas n und O Hb b COHb eingestellt hat. S nkt nun durch Hyperventilation der al olare CO Dru k, o ste gt entsp chend de al eolare O_2-Druck — ein Grosse d d rekt n die Ber chnung der COHb-Sattigung aus den Partialdrucken von CO und O des Atemsystems und einer Gle chge htsk nstanten ei g ht.

Fur die an 6 000 m H h adapti rte Ratte ist m t H lfe ines ndirekt n Verfah ens ein alveolar r CO Druck on 17 mm Hg rrechn t orden (TRIBUKAIT 196? d) Aus der etwa 40 Tage na h beendeter Hypoxi on 5 Tieren bestimmten Gleichgewichts-konstanten d e m t e n m Wert on 219 ± 15 8 mit dem Normalwert von 221 uberein st mmt ergibt s h f rner dass au h u d es m Ze tpunkt normale CO bzw O_2 Druckverhaltnisse o l egen Im ubrigen urd n unter Berucks chtigung der u von RAHN und OTIS (1948 besprochen n V ntilations erhaltnisse unter und nach Hypoxie folgende al eolar n CO Druck nach End der Hypoxieperiod ang nommen nach 2 T gen 20 mm Hg na h 5 T gen 24 mm Hg nach 10 Tagen 25 mm Hg nach 15 Tagen 28 mm Hg na h 25 T g n 30 mm Hg nach 0 Tag n 35 mm Hg

Hb Konz ntrat on und Hamatokrit aus denen s ch usammen mit dem Gesamt hamoglob n das Blut Plasma und Erythrozyten olumen berechn n wurden in der fruher angeg benen W ise bestimmt (TRIBUKAIT 1960 b)

Tabelle I. Gewicht und Blutwerte verschiedener Tiergruppen vor Hypoxie (a), in Hypoxie ent… Gruppen I und II befanden sich 43 Tage lang in Hypoxie, die Werte c entsprechen dem 36 … lang in Hypoxie, die Werte c und d entsprechen dem 43. bzw. 100. Tag nach beendeter Hypoxie

	n	Gewicht g	Tot. Hb g	g Hb / 100 g Gew	Rel. Hb g	Hct
I a	6	293 ± 4.3	1.97 ± 0.0 4	0.67 ± 0.071	13.01 ± 0.280	42.0 ± 1.12
b	6	275 ± 4.1	5.02 ± 0.178	1.83 ± 0.069	25.14 ± 0.721	78.2 ± 1.66
c	6	354 ± 2.0	2.43 ± 0.066	0.69 ± 0.019	11.02 ± 0.203	34.3 ± 0.18
II a	8	281 ± 4.4	2.06 ± 0.059	0.72 ± 0.023	12.73 ± 0.228	40.6 ± 1.00
b	8	259 ± 7.7	4.85 ± 0.230	1.87 ± 0.062	24.23 ± 0.339	75.2 ± 1.44
c	8	332 ± 11.2	2.27 ± 0.053	0.69 ± 0.014	11.13 ± 0.300	35.4 ± 0.53
III a	8	352 ± 8.9	2.50 ± 0.137	0.71 ± 0.027	13.35 ± 0.330	45.4 ± 1.00
b	6	325 ± 10.0	7.37 ± 0.407	2.27 ± 0.108	26.44 ± 0.422	85.3 ± 1.75
c	5	413 ± 14.6	3.01 ± 0.167	0.73 ± 0.023	11 0 ± 0.007	46.4 ± 1.86
d	5	436 ± 12.7	3.22 ± 0.145	0.74 ± 0.040	13.95 ± 0.405	43.2 ± 1.24

Ergebnisse

Die Resultate zweier Versuchsserien von 6 bzw. 8 Tieren, die eine weitgehende Übereinstimmung aufweisen, sind in Abb. 1 dargestellt. Aus Tab. I sind ferner die Daten vor Hypoxie, nach 43 tägiger Hypoxie entsprechend 6000 m Höhe sowie 36 bzw. 43 Tage nach Ende der Hypoxieperiode ersichtlich.

43 tägige Hypoxie führt zu einer markanten Polyzythämie: das Gesamthämoglobin ist um etwa 150 % gestiegen, Hb-Konzentration und Hämatokrit um etwa 100 %. Bei einem Abfall des Plasmavolumens um 20—25 % ist das Blutvolumen um 25—30 % hochsignifikant gestiegen ($p < 0.001$). Der Abfall des Plasmavolumens ist ebenfalls statistisch signifikant ($p < 0.001$), auch wenn das Plasmavolumen auf das Körpergewicht bezogen wird (ml Plasmavolumen/100 g Körpergewicht: $p = 0.01$—0.001), obwohl ebenfalls das Körpergewicht hochsignifikant abgefallen ist ($p < 0.001$).

Nach Übergang von Hypoxie auf Meereshöhe (Tag 0 der Abb. 1) steigt das Körpergewicht rasch an. Auf diese Veränderungen wird unten noch näher eingegangen.

Das Gesamthämoglobin der einen Gruppe erreicht nach rund 29 Tagen ein neues Niveau, das der anderen Gruppe nach etwa 35 Tagen. Diese Werte stimmen gut mit den Normalwerten von gleichgrossen Tieren dieses Stammes überein (TRIBUKAIT 1960 a). Diese sind in Abb. 1 gestrichelt eingezeichnet worden. Der Charakter der beiden Kurven entspricht am ehesten einem exponentiellen Verlauf. Dieser wird bei semilogarithmischer Darstellung deutlich, auf deren graphische Wiedergabe hier jedoch verzichtet worden ist. Die Gleichungen der Regressionslinien und deren Korrelationskoeffizienten lauten: $y = -0.0113x + 0.6983$ $r = -0.996$ und

$y = -0.0098x + 0.7013$ $r = -0.993$

sprechend 6000 m Höhe (b) und nach Abschluss der Hypoxieperiode (c, d). Die Tiere der c bzw. d 43. Tag nach beendeter Hypoxie. Die Tiere der Gruppe III befanden sich 48 Tage
n = Anzahl der Tiere

Rel. Hb g% / Hct × 100	Blutvol. ml	ml Blutvol./100 g Gew	Ery vol. ml	ml Ery vol./100 g Gew	Pl vol. ml	ml Pl. vol./100 g Gew
31.0 ± 0.16	20.2 ± 0.41	6.9 ± 0.12	6.4 ± 0.27	2.2 ± 0.08	13.8 ± 0.22	4.7 ± 0.07
3[illegible].2 ± 0.46	26.6 ± 0.73	9.7 ± 0.31	15.6 ± 0.58	5.7 ± 0.23	11.0 ± 0.43	4.0 ± 0.17
32.1 ± 0.63	29.4 ± 0.86	8.3 ± 0.24	7.6 ± 0.23	2.2 ± 0.06	21.8 ± 0.64	6.2 ± 0.17
31.4 ± 0.22	21.6 ± 0.81	7.6 ± 0.32	6.6 ± 0.19	2.3 ± 0.07	15.1 ± 0.68	5.2 ± 0.26
32.2 ± 0.44	26.6 ± 1.09	10.3 ± 0.27	15.0 ± 0.72	5.8 ± 0.23	11.6 ± 0.48	4.5 ± 0.10
31.5 ± 0.74	27.3 ± 0.82	8.2 ± 0.24	7.2 ± 0.26	2.2 ± 0.05	20.0 ± 0.60	6.1 ± 0.20
29.5 ± 0.75	25.1 ± 1.53	7.1 ± 0.32	8.5 ± 0.56	2.4 ± 0.13	16.6 ± 1.02	4.7 ± 0.[illegible]1
31.0 ± 0.25	37.1 ± 1.64	11.4 ± 0.47	23.8 ± 1.39	7.3 ± 0.36	13.3 ± 0.48	4.1 ± 0.19
25.4 ± 1.03	34.3 ± 1.91	8.3 ± 0.03	12.0 ± 0.91	2.9 ± 0.19	22.3 ± 1.23	5.4 ± 0.15
3[illegible].3 ± 0.26	30.8 ± 1.29	7.1 ± 0.46	10.0 ± 0.[illegible]0	2.3 ± 0.15	20.8 ± 0.91	4.8 ± 0.34

Der Abfall der Hb-Konzentration und des Hämatokrit bis zum 29. bzw. 36. Tag entspricht nicht ganz dem des Gesamt-Hb. Das bedeutet, dass das Blutvolumen gleichfalls langsam sinkt und zu diesem Zeitpunkt Normalwerte erreicht. Das Plasmavolumen steigt ziemlich rasch und unterscheidet sich bei der einen Gruppe schon nach 9 Tagen nicht mehr vom Normalwert, bei der anderen nach 23 Tagen. Überraschend fallen dann plötzlich die relativen Blutwerte zwischen 29. und 36. Tag bzw. 36. und 43. Tag auf eine Hb-Konzentration von etwa 11 g % und einen Hämatokrit von etwa 34 % . Diese weichen ganz beträchtlich von den Normalwerten ab. Entsprechend steigen Blutvolumen und Plasmavolumen hochsignifikant ($p < 0.001$). Die mittlere Hb-Konzentration der Erythrozyten, ausgedrückt durch das Verhältnis der Hb-Konzentration zum Hämatokrit, ist dabei unverändert.

Während in diesen beiden besprochenen Versuchsserien eingehender die Readaptation der Blutwerte bis zur Normalisierung des Gesamthämoglobin untersucht worden ist, ist in der dritten Versuchsserie vor allem der weitere Verlauf verfolgt worden. Dabei wurden grössere Tiere in der Absicht gewählt, von Wachstumsveränderungen unabhängiger das Blutvolumen verfolgen zu können. Das war allerdings nicht der Fall. Ferner wurden die Blutwerte nur vor Hypoxie, 48 Tage nach Hypoxiebeginn sowie erst wieder 43 Tage nach Ende der Hypoxieperiode bestimmt, um die Möglichkeit einer Beeinflussung der Tiere durch zu häufige Blutentnahmen (Nachblutungen, Infektionen) auszuschliessen. Leider starben im Laufe der Zeit 3 von 8 Tieren. Da aber die Mittelwerte aller Ausgangswerte unabhängig davon waren, ob sie von 5, 6 oder 8 Tieren berechnet wurden, wurden jeweils alle verfügbaren Werte verwendet.

Die Resultate sind Abb. 2 sowie Tab. I zu entnehmen. Die Polyzythämie ist nach 48-tägiger Hypoxie mit einem Anstieg des Gesamthämoglobin um rund

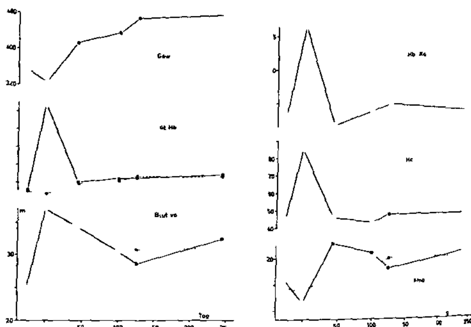


Abb. 2. Gewicht und Blutwerte einer Gruppe von Ratten vor Hypoxie (Mittelwerte von 8 Tieren), nach 48tägigem Aufenthalt in Hypoxie entsprechend 6000 m Höhe (Mittelwerte von 6 Tieren) und nach Übergang auf Meereshöhe (Tag 0: Mittelwert von 5 Tieren). Die Normalwerte für Gesamthämoglobin, Blut- und Plasmavolumen für Tiere entsprechender Größe sind gestrichelt eingezeichnet

200 % der relativen Blutwerte um etwa 100 % und des Blutvolumens um rund 50 % beträchtlich.

43 Tage nach Ende der Hypoxieperiode entspricht das Gesamthämoglobin genau dem Normalwert von Kontrolltieren dieses Gewichts. Im Verlauf der weiteren 200 Tage steigt dann das Gesamthämoglobin etwas, aber auch nur entsprechend dem Gewichtszuwachs. Die Hb-Konzentration ist am 43. Tag mit 11,7 g % ähnlich niedrig wie auch bei den beiden ersten Versuchsgruppen. Der Hämatokrit unterschreitet dagegen aber nicht den Normalwert. Daraus resultiert eine abnorm niedrige mittlere Hb-Konzentration der Erythrozyten ($p < 0{,}001$). Im weiteren Verlauf weichen Hb-Konzentration und Hämatokrit nicht mehr von der Norm ab. Blut- und Plasmavolumen sind ebenso wie bei den ersten beiden Versuchsgruppen am 43. Tag sehr hoch, dabei ist das auf das Körpergewicht bezogene Blutvolumen bei allen drei Versuchsgruppen völlig gleich. Das Blut- und Plasmavolumen sind dann vom 100. Tag an wieder praktisch normal.

Die Veränderungen des Körpergewichts der drei Versuchsgruppen während und nach Hypoxie sind in Abb. 3 zusammen mit dem durchschnittlichen Wachstumsverlauf normaler Tiere zusammengestellt. Dieser wurde aus dem mittleren Zuwachs von hundertzehn 200—450 g schweren Tieren berechnet.

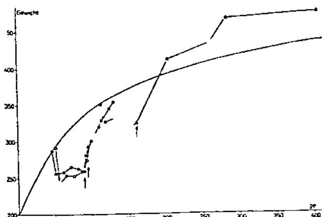


Abb. 3. Änderungen des Gewichts von 3 Tiergruppen während und nach Hypoxie entsprechend 6 000 m Höhe. Mittelwerte von jeweils 5—8 Tieren. Die Pfeile kennzeichnen das Ende der Hypoxieperiode. Der gestrichelt angedeutete Kurvenverlauf der dritten Gruppe während Hypoxie entspricht einem Erfahrungswert. Die ausgezogene Kurve gibt den von 110 Tieren dieses Stammes gefundenen durchschnittlichen Gewichtsanstieg wieder.

In Übereinstimmung mit den Erfahrungen an einem grösseren Material (TRIBUKAIT 1962 a) ist das Körpergewicht der beiden ersten Gruppen nach 2 tägiger Hypoxie um 10—15 gesunken. Da von der dritten Gruppe mit einem Ausgangsgewicht von 350 g der Abfall nicht gemessen worden ist, wurde ein Erfahrungswert eingesetzt und der Kurvenverlauf nur punktiert angedeutet. Im weiteren Verlauf der Hypoxie steigt das Gewicht nur geringfügig oder nicht. Mit dem Ende der Hypoxieperiode steigt das Gewicht sprunghaft. Der Wachstumsverlauf dieser Tiere entspricht jetzt ganz und gar nicht ihrem Alter. Aber auch wenn man vom Gewicht bei Ende der Hypoxieperiode ausgeht, ist der Gewichtszuwachs dieser Tiere verglichen mit dem von Normaltieren höher. Das wird besonders deutlich bei der dritten Gruppe, deren Endgewicht das Durchschnittsgewicht von Normaltieren um etwa 40 g übersteigt.

Diskussion

Hämatologische Studien der Umstellung eines höhenadaptierten Organismus auf Meereshöhe können unter zwei verschiedenen Aspekten vorgenommen werden: 1. im Hinblick auf Fragen wie Zellabbau, Hemmung der Zellproliferation, Eisenstoffwechsel u. a. — im wesentlichen also morphologisch-biochemische Probleme; 2. im Hinblick auf das offenbar homoiostatische Gleichgewicht der Erythropoiese — ein rein funktionelles Problem. Beide Problemkreise sind untrennbar miteinander verbunden und stellen nur zwei Seiten ein und desselben Fragenkomplexes dar.

Die Zahl der Studien über die Readaption von höhenadaptierten Organismen an Meereshöhe ist relativ spärlich. Im wesentlichen ist dabei die Frage diskutiert worden, inwieweit der Abfall der Erythrozytenzahl a) durch einen

aktiven gesteigerten hämolytischen Prozess und b) durch eine Hemmung der Zellneubildung bei normalem Erythrozytenabbau verursacht wird

Untersuchungen des Zellumsatzes mit Hilfe von Gesamthämoglobin und Bilirubin bzw Urobilinogenausscheidung haben dabei zur Vorstellung eines beschleunigten Zellabbaus bei gleichzeitiger Hemmung der Zellneubildung geführt (MERINO 1950 REISSMANN BURKHARDT und HOLLSCHER 1952) Gegen diese Resultate können jedoch Einwände erhoben werden Nach LONDON *et al* (1948 1950) stammen nur etwa 70 % des Bilirubin vom Erythrozytenabbau der Rest von Cytochrom Katalase Myoglobin und einer unbekannten Quelle die mit der Hb Bildung in Zusammenhang stehen soll Ferner kann eine veränderte Leberfunktion unter Hypoxie bedeutungsvoll sein Die Resultate von Fe Umsatzbestimmungen haben HUFF *et al* (1951) in gleicher Weise gedeutet

Direkte Messungen der Erythrozytenlebenszeit beim Übergang von Höhe auf Normaldruck haben andererseits keinen oder nur einen äusserst unbedeutenden gesteigerten Abbauprozess wahrscheinlich gemacht (FRYERS und BERLIN 1952 REYNAFARJE LOZANO und VALDIVIESO 1959) *Letztere Untersuchungen* zeichnen sich u a besonders dadurch aus als sie an vollakklimatisierten Höhenbewohnern der Anden ausgeführt worden sind Damit kann eine homogene Verteilung des Erythrozytenalters im Blut angenommen werden — ein verständlicherweise wichtiger Punkt bei der Beurteilung des Erythrozytenabbaus

Vom Standpunkt der Regulation der Erythropoiese aus betrachtet kommt einem gesteigerten Zellabbau unter diesen Bedingungen eine nicht unbeträchtliche theoretische Bedeutung zu Ein aktiver zellabbauender Prozess bei einem erhöhten Sauerstoffgehalt des Blutes bedeutet nämlich u a dass dieser bei normalen oder verringertem O Gehalt gehemmt werden muss Es ist in diesem Zusammenhang von Interesse dass die Erythrozytenlebenslänge bei der Entwicklung der Höhenpolyzythämie d h einem verringerten O Gehalt des Blutes normal eventuell etwas verkürzt ist keinesfalls aber verlängert (FRYERS und BERLIN 1952 REYNAFARJE BERLIN und LAWRENCE 1954 BERLIN REYNAFARJE und LAWRENCE 1954)

Die vorliegenden Untersuchungen konnen nicht zu einer Klärung der *erörterten Fragen beitragen Der Abfall der* Gesamthämoglobinmenge auf ein neues Niveau innerhalb von etwa 30 Tagen entspricht zwar gerade der Hb Menge die bei unterdrückter Hb Bildung und einer Erythrozytenlebenslänge von 60 Tagen normalerweise abgebaut wird Da aber unbekannt *ist ob* während dieser Zeit noch Hb gebildet wird und da gerade zu diesem *Zeitpunkt* das Ende der Lebenszeit der während der ersten Zeit in Hypoxie gebildeten Erythrozyten *erreicht* sein *dürfte sind Aussagen* über die *Art des* Abfalls nicht möglich Bemerkenswert ist dabei der offenbar exponentielle Kurvenverlauf der auch beim Zellabbau unter Normalbedingungen beobachtet worden ist (FORSSBERG und TRIBUKAIT 1962) und einer Resorptionskurve sehr ähnlich ist

Für das Problem der homoiostatischen Regulation der Erythropoiese hat das Verhalten der Hb Konzentration und des Blutvolumens während der nachhypoxischen Periode ein spezielles Interesse Da unter Hypoxie nicht nur die Hb Konzentration sondern auch das Blutvolumen und damit eng verbunden die Kapillarisation des Gewebes ansteigt sind in der Readaptationsperiode die Voraussetzungen einer über das Normale hinausgehenden O_2-Versorgung des Gewebes gegeben Wird die Funktion der Erythropoiese durch Prozesse gesteuert die von der Grösse der O_2 Versorgung des Gewebes abhängig sind ist durch eine verbesserte O Versorgung des Gewebes infolge dieser erhöhten Kapillarisation ein Absinken der O Kapazität des Blutes unter den Normalwert hinaus denkbar

Ein solcher Mechanismus setzt allerdings voraus dass 1 die erhöhte Kapillarisation auch unter normalen O_2 Druck Bedingungen tatsächlich funktionell wirksam ist Das bedeutet dass diese Kapillaren auch nach der Readaptation in etwa gleichem Umfang durchblutet werden wie unter Hypoxie 2 muss auch das für die Erythropoiese in diesem Zusammenhang entscheidende Gewebe eine erhöhte Kapillarisation aufweisen 3 müssen die Mechanismen (Fermentsysteme etc) die auf Hypoxie zu einer erhöhten Erythropoiese führen bei hohem O_2 Druck in entgegengesetztem Sinne wirksam werden

Nach den Untersuchungen von HUERKAMP und OPITZ (1950) ist auch längere Zeit nach der Readaptation mit einem anatomischen Fortbestand der erhöhten Kapillarisation zu rechnen Das Auge des Kaninchens zeigte 82 Tage nach Ende einer 6-monatigen Hypoxieperiode in 6 000 m Höhe eine um etwa 50 % vermehrte Kapillarisation Auf eine länger anhaltende funktionelle Wirksamkeit dieser erhöhten Kapillarisation lässt sich aus den Versuchen CAMPBELL s (1927) schliessen erst etwa 40 Tage nach Ende einer 40 tägigen kräftigen Hypoxieperiode hatte sich beim Kaninchen der subcutane bzw intraperitoneale O Druck dem Normalwert genähert Über Art und Sitz des mit der Erythropoiese im Zusammenhang stehenden Gewebes lassen sich bisher keine sicheren Aussagen machen Hinsichtlich der O Druck Abhängigkeit der Leistung biologischer Systeme sei nur an Hb erinnert

Das Auftreten einer Anämie bei der Readaptation an normale O_2 Druck Bedingungen das eventuell die Vorstellung eines funktionellen Zusammenhangs zwischen der Grösse der Kapillarisation und der O Kapazität des Blutes stützen kann ist verschiedentlich beschrieben worden TYLER und BALDWIN (1933/34) sowie GORDON und KLEINBERG (1937) haben an Ratten bzw Meerschweinchen HURTADO MERINO und DELGADO (1945) sowie REYNAFARJE *et al* (1959) bei Bewohnern der Anden die Jahrzehnte in über 4 000 m Höhe gelebt hatten niedrige Hb Konzentrationen gefunden Gesamt Blutvolumen Bestimmungen bei einigen Höhenbewohnern lassen eine vorübergehende absolute Anämie erkennen der eine langanhaltende relative Anämie folgt (REYNAFARJE *et al* 1959)

In den eigenen Versuchen wird ebenfalls eine Anamie gefunden Diese ist aber nur relativ d h bei normalem Gesamthamoglobin ist lediglich das Plasmavolumen gestiegen Damit scheidet die Moglichkeit einer zeitweiligen Insuffizienz des erythropoietischen Gewebes als Ursache der Anamie aus Sie entwickelt sich ferner erst plotzlich nach der Normalisierung des Gesamt Hb etwa 30 Tage nach Ende der Hypoxie Bis zu diesem Zeitpunkt fallt die Hb Konzentration relativ langsamer als das Gesamt Hb womit auch das Blutvolumen sinkt Das Blutvolumen ist bis dahin zwar hoher als zu Beginn des Versuchs Da aber inzwischen die Tiere gewachsen sind was das Bild kompliziert, ist es normal in Relation zum Korpergewicht Die Moglichkeit dass im Zusammenhang mit den haufigen Blutentnahmen Nachblutungen oder Infektionen zu dieser spat auftretenden Anamie gefuhrt haben kann durch die Resultate des 3 Versuchs ausgeschlossen werden Dabei waren nur in 40—50 tagigen Abstanden Blutproben entnommen worden Der Hamatokrit dieser Gruppe entsprach nicht ganz der Hb Konzentration — eine Erscheinung die gelegentlich auch wahrend Hypoxie bei alteren Tieren beobachtet worden ist (TRIBUKAIT 1962 a)

Bei der hier besprochenen Anamie handelt es sich also um eine reine Verdunnungsanamie ohne Beteiligung des Gesamthamoglobin Bei zwei anderen untersuchten Anamieformen der akuten Blutungsanamie (TRIBUKAIT 1960 c) und der sich langsam entwickelnden Anamie nach Rontgenbestrahlung (TRIBUKAIT nicht veroffentlicht) fiel die Hb Konzentration nur entsprechend dem Abfall des Gesamt Hb Das Blutvolumen war also unverandert Es ist wohl denkbar dass die Voraussetzung fur die relative Anamie nach Hypoxie das unter Hypoxie angestiegene Gefassystem ist

Über den weiteren Verlauf der relativen Anamie sind hier keine sicheren Aussagen moglich 100 Tage nach Ende der Hypoxie werden zwar normale Blutwerte gefunden Da aber das Gewicht der Tiere inzwischen angestiegen ist, muss offen bleiben ob die Tiere gewissermassen nur in ihr grosseres Gefasssystem hineingewachsen sind oder bei unverandertem Gewicht weiter anamisch geblieben waren

Im ganzen gesehen scheint zwar nach den Angaben der Literatur und den eigenen Untersuchungen zeitweilig eine Anamie wahrend der Ruckadaptation an normalen O Druck auftreten zu konnen Daraus jedoch auf einen funktionellen Zusammenhang zwischen dem Grad der Kapillarisation des Gewebes und der Grosse der O Kapazitat des Blutes zu schliessen ist so lange nicht sicher moglich als vor allem die kapillare Durchblutung einen unbekannten Faktor darstellt

Der rasche Gewichtszuwachs nach Hypoxie zeigt dass die hypoxische Wachstumshemmung nicht auf einer irreversiblen Schadigung der Tiere beruht. Ein ahnliches Verhalten ist ebenfalls nach Hypoxie (ALTLAND 1949) vor allem aber Mangelernahrung verschiedener Art (CLARKE und SMITH 1938 JACKSON 1939 v BERTALANFFY 1951) beschrieben worden Es ist auffallig dass

die alteren Tiere hohere Endgewichte als der Durchschnitt von Normaltieren erreichen Es lasst sich hier nicht entscheiden ob das auf einer Auswahl be sonders wachstumskraftiger Tiere beruht oder ob spezielle Bedingungen nach Hypoxie ein ubernormales Wachstum bewirken

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Behaviour and Monoamine Levels During Long-Term Administration of Reserpine to Rabbits

By

JAN HAGGENDAL and MARGIT LINDQVIST

Rece d 14 S ptember 1962

Abstract

HAGGENDAL J and M LINDQVIST *Behaviou and monoam ne levels duri g lo term adm nistration f e pine t abbit* Acta phys ol scand 1963 *57* 431—436 — Rabb ts were injected with reserpine (0 1 and 0 2 mg per kg subcutan ously) daily for weeks Noradr naline dopamine and 5 hydroxytryptamine were determined in the b ain no adr naline in the hea t and adrenaline in the adrenals Most of the monoamines disappeared from th tissues during the first few w ks Almost normal behaviour was observed in animals with very lo v monoamine le ls Thus the m jor part of the monoamine sto es could be remo ed vith but light functional changes The data sug gested however a correlation between the r maining small fraction and behaviour

In the study of the monoamines in the central nervous system the behaviour of the animals has been correlated with increased or decreased levels of the brain monoamines after administration of certain drugs Much attention has been paid to the effects of reserpine and the sedation of the animals has been correlated with decreased levels of noradrenaline dopamine and 5 hydroxy tryptamine (5 HT)

Many investigators regard this sedation to be caused by a lack of one or several of the monoamines (CARLSSON *et al* 1957 OLDS 1959) According to BRODIE and coworkers however the sedation is caused by an excess of 5 HT liberated from the stores (see SHORE *et al* 1957) An overt sedation has been associated with a depletion of about 50 per cent of the normal 5 HT v rabbit brain (COSTA *et al* 1962)

Most of the studies on the relationship between behaviour and brain monoamine levels seem to have been performed with one or a few large doses of reserpine

Some experiments on behaviour only have been made after small daily doses of reserpine (PLUMMER et al 1954)

In the present investigation the effects of small daily doses of reserpine were studied with respect to behaviour and brain levels of noradrenaline dopamine and 5 HT

Methods

White female rabbits weighing about 1 5 kg received daily subcutaneous injections of reserpine One group received 0 1 mg per kg for the first 15 days and thereafter 0 2 mg per kg (series I) Another group received 0 2 mg per kg throughout the experiment (series II) A single dose of reserpine 0 2 mg per kg was given to 4 rabbits Ampoules of Serpasil (Ciba) containing 2 5 mg per ml diluted with 5 per cent glucose were used Every rabbit received 1 0 ml per kg of body weight Control rabbits were injected with the corresponding volume of the vehicle (Ciba) The animals were kept in a quiet room at + 22 C

The rabbits were carefully observed several times a day for signs of sedation (decreased motor activity and lowered responsiveness to stimuli) posture miosis and ptosis

The animals were killed by air embolism in series I 20 hours and in series II 4 or hours after the last injection of reserpine The rabbits which received a single dose were killed after 4 or 28 hours

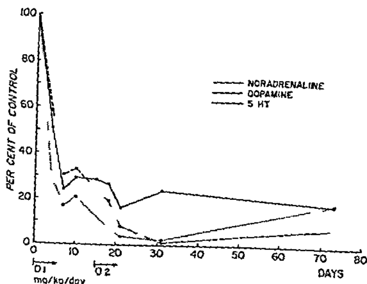
The brain heart and adrenals were immediately removed and homogenized with 0 4 N perchloric acid Adrenaline and noradrenaline were determined according to the method of BERTLER, CARLSSON and ROSENGREN (1958) and dopamine according to CARLSSON and WALDECK (1958) modified according to CARLSSON and LINDQVIST (1962) 5 HT was estimated by the method of BERTLER (1961) except that the perchloric acid residue was reextracted once with 0 4 N perchloric acid (2 ml per g tissue)

Results

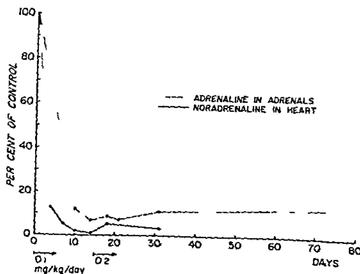
Behaviour of the rabbits

When the animals in series I were observed *4 hours after the daily injection* of 0 1 mg reserpine per kg of body weight increasing reserpine signs appeared during the first few days sedation typical posture miosis and ptosis On the 5th day the effects of this dose of reserpine seemed to decline During the second week the animals looked almost normal On the 16th day the daily dose was increased to 0 2 mg per kg of body weight After every daily dose clearcut reserpine signs reappeared which remained nearly the same from the 4th week and throughout the experiment

The reserpine signs were generally weaker *20 hours after the daily injection* than after 4 hours This was especially true during the latter part of the experiment After the 20th day the animals hardly could be distinguished from the controls



Fg 1 Noradrenaline dopamin and 5 hydroxytryp amine in abb t brain during long term adminisi ation f serpin



Fg 2 N radrenaline in rabbi hea t and adrenaline n rabb t adrenals during l ng term adm nistrat on f reserpine

except for miosis So there was definite evidence of adaptation as the animals during the first week of treatment showed clearcut signs of reserpine action even 20 hours after each injection of the smaller dose.

Table I *Noradrenaline dopamine and 5-hydroxytryptamine in rabbit brain 4 and 28 hours after injection of reserpine*

	4 hours				28 hours			
	NA	DA	5 HT	No of animals	NA	DA	5 HT	No of animals
Reserpine 0.2 mg/kg to rabbits pretreated with reserpine								
Experiment 1	0.010	0.000	0.032	1	0.020	0.011	0.077	1
Experiment 2	0.011	0.036	0.027	1	0.030	0.075	0.128	1
Reserpine 0.2 mg/kg								
No pretreatment	0.17 ± 0.059	0.33 ± 0.079	0.16 ± 0.043	4	0.13 ± 0.019	0.32 ± 0.029	0.10 ± 0.014	4
Normal	0.35 ± 0.023	0.57 ± 0.027	0.43 ± 0.0[illegible]1	4	–	–	–	–

Single values expressed in $\mu g/g$ tissue
Values are means ± standard error of the mean, expressed in $\mu g/g$ tissue.

When the animals had received reserpine 0.2 mg per kg daily for several weeks (series II) the signs were clearcut after 4 hours but after 28 hours the animals looked almost normal as in series I

The normal increase of body weight stopped after the administration of 0.2 mg per kg had started but after a few weeks the weights of the rabbits started to increase again (data not shown)

Levels of noradrenaline dopamine and 5-hydroxytryptamine Series I The brain monoamines of the rabbits in series I are shown in Fig 1 as per cent of control values The control values did not alter during the experiment

After the daily administration of 0.1 mg reserpine per kg of body weight for one week the brain monoamines were low about 20 to 30 per cent of the control values The values one week after the dose was increased to 0.2 mg per kg were still lower for noradrenaline less than 5 per cent for dopamine less than 10 per cent and for 5 HT less than 20 per cent of the control values The values remained low during the rest of the experiment *i e* more than 10 weeks from the start of the administration of reserpine

In the heart there was a rapid decrease of the noradrenaline level and it remained very low throughout the experiment In the adrenals the adrenaline content was strongly reduced but tended to increase slightly towards the end of the experiment (Fig 2)

Series II Rabbits which had received reserpine 0 2 mg per kg daily for several weeks were studied with respect to behaviour and brain monoamine levels 4 and 28 hours after the last injection After 4 hours they showed clear cut reserpine signs and extremely low brain monoamine levels the lowest so far observed in this investigation Twentyfour hours later when the animals looked almost normal the brain levels had increased again (Table I)

The table also shows the brain monoamine levels of rabbits 4 and 28 hours after a single dose of reserpine 0 2 mg per kg The behaviour of these animals did not differ markedly from that of the pretreated animals described above The brain monoamine values 4 and 28 hours after the injection were of about the same order of magnitude with perhaps a slight decrease of the 28 hour values and generally much higher than those of the pretreated animals

Four hours after the last injection the adrenals of the pretreated animals contained very small amounts of adrenaline about 2 per cent of the control values After 28 hours there was perhaps a slight increase

Discussion

The results of series I show no correlation between the behaviour of the animals and their brain monoamine levels During the first week of administration the most clearcut reserpine signs and at the same time the highest monoamine levels were observed From the 4th week and onwards the animals looked almost normal but the monoamine levels were very low

In the pretreated animals of series II there seemed to be a correlation between the behaviour of the animals and their very low brain monoamine levels both behavioural and biochemical effects were more pronounced after 4 than after 28 hours

The results may be explained by the monoamines being present in the brain in two main fractions one large fraction perhaps stored monoamines which may be reduced by reserpine without any effects on the behaviour and one smaller fraction which may also be reduced but in this case with effects on the behaviour

Changes in sensitivity to the monoamines or in the rate of their synthesis may also be involved and explain the adaptation to reserpine

In this laboratory MARKIEWICZ (1963) has extended this experiment to mice The results obtained are in general agreement with our results With the doses used however the adaptation was more evident in the mice than in the rabbits The catechol amines in the mouse adrenals increased markedly in the latter part of the experiment and the adrenaline level returned almost to the normal value This increase could hardly be detected in the adrenals of the rabbits

The present results appear to call for a re-evaluation of earlier investigations in which attempts have been made to link sedation to a definite level of one monoamine

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Acta physiol. scand 1963 57 437—445

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Sinus Nerve Stimulation in the Chloralose Anesthetized Cat Effect on Blood Pressure, Heart Rate, Muscle Blood Flow and Vascular Resistance

By

THOMAS KILLIP III M D

R ed 21 S pt mber 1962

Abstract

KILLIP THOMAS *S nus nerv t mulati n n the hlo al nesthets d at* Acta phys ol scand 1963 57 437—445 — In the chloralos anesthetized cat sho t durat on stimul (0 05—0 1 ms c) of the sinus nerve produce a presso esponse This p epa ation as util zed to exam ne the relation betwe n fr quency of d rect chemo eceptor f ber stimulation and change in blood pressure heart rat muscle blood flo and muscle ascula resistan e Blood pressure hea t rate and muscl ascula esistance ncreased during s nus nerve stimul ation p oport on l to th f equ ncy Muscl blood flow emain d es sentially constant desp te the pressu e rise Responses re detected at 0 5 cp Maximum respons occurred at 16—20 ps The range of f equenc espons fo ascula es tanc nd heart ate s milar to that obtained by t mulat ng ympathet cs d th Thus both limbs of a card ascula flex a ha s mila f equenc response cha acte stics Th l k l hood that the data obtain d r p esents an app oximat on of th h moc pto responses wh ch n other prepar at ons ar masked by p dom nan of baroceptor fun tion is discus sed

As is well kno n stimulation of the carotid body chemoreceptors by indirect means such as decreasing arterial pO increasing arterial pCO or injecting small amounts of certain drugs produces a rise in blood pressure and an increase in vascular resistance in different vascular beds (HEYMANS and NEIL 1958) Changes in heart rate may also occur Attempts to study the response to direct electrical stimulation of the chemoreceptor fibers have been limited by the fact

that the sinus and aortic nerves contain both baroreceptor and chemoreceptor fibers When the nerve is stimulated directly baroreceptor depressor function usually predominates and blood pressure falls the heart slows and there is inhibition of sympathetic discharge (BRONK et al 1936)

NEIL REDWOOD and SCHWEITZER (1949) have shown that in the cat anesthetized with chloralose stimulation of the sinus nerve with impulses of short duration has a pressor effect suggesting that chemoceptor function is predominant The present experiments utilizing the observation of NEIL et al were designed to obtain quantitative data in the form of stimulus response curves on the effect of electrical stimulation of the chemoceptor fibers in the sinus nerve upon blood pressure heart rate and muscle vascular resistance It was postulated that this information obtained by stimulating the afferent limb and recording from the efferent limb of a cardiovascular reflex arc when compared to data of others obtained by stimulating and recording from the efferent limb would provide insight into the central control of the circulation

Methods

Experiments were performed on 12 cats anesthetized with 70 mg chloralose/kg intravenously A supplementary dose of 2 —35 mg of chloralose/kg was given one half hour prior to obtaining the experimental data If the supplementary dose were not given stimulation of the sinus nerves frequently produced a mixed or a depressor response

Both sinus nerves were exposed from the carotid body to their junction with the glosso pharyngeal nerve and cut close to the carotid body The nerves were repeatedly washed with saline to prevent drying and then carefully blotted To eliminate the buffering effect of thoracic baroreceptors and chemoreceptors the vago-sympathetic trunks and the aortic nerves were cut in the neck The animals were maintained on positive pressure respiration at a frequency of 16—20 per minute adjusted prior to section of the vagi to just depress spontaneous ventilation

The sinus nerves were laid across small silver wire bipolar electrodes and stimulated with a Grass S4 square wave stimulator Voltage (4 or 6 volts) and pulse duration (0 05 or 0 1 msec) were adjusted to give maximum pressor response Frequency varied between 0 5 and 50 impulses per second Stimulation with a pulse duration of 1 0 msec or longer gave a mixed or a depressor response Stimulus duration varied from 30 to 90 seconds depending on the time required to reach a maximum response

Arterial blood pressure was recorded utilizing Statham strain gauges and a Grass direct writing polygraph Muscle blood flow was recorded with a drop recorder (LINDGREN 1958) which was enclosed in a water jacket maintained at 38 C and connected to an ordinate writer (GOLDSCHMIDT and LINDGREN 1962) The ordinate writer is so constructed that the height of the marking pen above the baseline is inversely proportional to blood flow Heart rate was recorded by the method of GOLDSCHMIDT and LINDGREN (1962) A heat lamp maintained body temperature 37—38 C

Muscle blood flow was recorded from a hind limb which had been skinned with a cautery knife and the skin reapproximated with clips A tight ligature was placed about the ankle to exclude the paw circulation A specially designed screw clamp was was inserted either in the aorta or the iliac artery When the iliac artery was used all other branches at the bifurcation of the aorta were ligated The outflow tubing of the

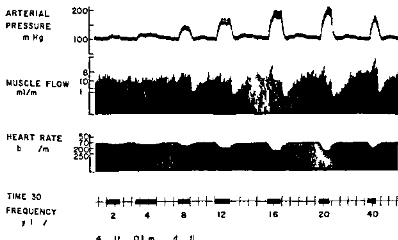


Fig 1 Effect of sinus n rve st mul t on f reas g f quency in the hl alose an tl tized cat on blood p essu e muscl blood flow and h art at Maximum effect n blood p essur a d he rt at oc urs at 20 cps St mulat n t 40 cp is p amax mal M l blood fl w remains essentially nstant during th t m lat on Immed t l ft timulus is w thdrawn th e is a triking incr asing in fl w lasting a min t or m

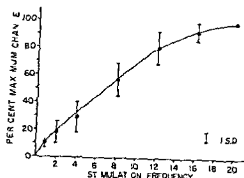
drop hamber was ins ted in the f moral artery and the nflow tub ng connect d to the screw clamp Clotting was pre nted by the adm n str tion of heparin Arterial pressur s wer recorded prox mal (syst mic) and d stal (perfus on) to the clamp Manual adjustment of the clamp permitted ma ntenan of a constant perfus on p essure desp te changing systemic pressure Muscle esistan as calculated utiliz ng the Po s uill equation from the change in flow h n perfus on pressu e as mainta ned constant

St mulus response curves of the ff ct of nus nerv timulation on arterial pressure and he rt rate w e obta ned indep ndently f om the data on muscle flo and resistanc for du ng the latter p ocedures perfus on pressure as h ld constant For ach experi ment data on change in heart rat blood pressure muscle blood flo and mus le vascular resistance were compared at each timulat on f equency n t rms of the per nt of maximum change obs rv d

Du ng each series of st mulat ons blood pressure was ma nta ned at a constant control lev l between 100—130 mm Hg by administration of Dextran as needed When muscle blood flow was determ n d under constant p rfusion pressu perfusion pressur was ma nta ned constant at 80 90 or 100 mm Hg

Results

Stimulation of the sinus nerve in the chloralose anesthetized cat produced an increase in blood pressure and heart rate which was proportional to the frequency of stimulation (Fig 1) The curves have the familiar rectangular hyperbolic form When perfusion pressure was held constant stimulation of the sinus nerve resulted in a decrease in muscle blood flo and therefore an in crease in calculated vascular resistance in proportion to the stimulation fre quency



F g 2

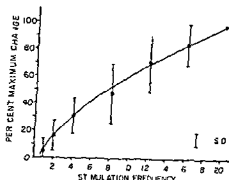
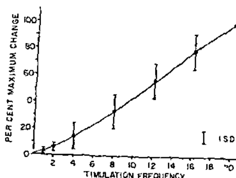


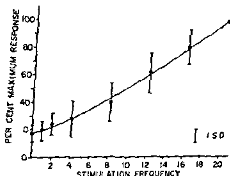
Fig 3

Fig ? Systemic arterial p essure change dur ng sinus nerve stimul t on Ho izontal bars ind cate 1 standard deviation of mean.

F g 3 Change in l eart rate during sinus r erve stimulat on



F g 4



F g 5

I g 4 Chang n n uscle vascular esistance with constant perfus on press e during sinus nerve timulati n.

F g 5 Muscl es tance dur ng inus n rv stimulat n Maximum res tance bserved equated to 100 units Co trol muscle esistance a e aged 18 un ts Sinus r erve timulation p oduced mo e than fi e fold ncreas in es stance

The stimulus response curves for change in blood pressure heart rate and resistance are shown in Fig 2—4 The curves were plotted by calculating the changes in the functions measured at each stimulation frequency as a *percent* of the maximum change observed In Fig › the change in muscle vascular resistance is also plotted in absolute terms the maximum resistance observed with stimulation is termed 100 units From this figure it may be seen that the average control muscle resistance before stimulation was 18 % of the maximum

Maximum responses for all parameters measured occurred at frequencies of 16—20 cycles per second Stimuli greater than 30 cps were supramaximal Detectable re ponses were frequently observed at frequencies as low as 0 5 cps

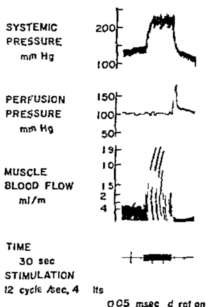


Fig 6 Systemic pressure and muscle blood flow during sinus nerve stimulation with constant muscle perfusion pressure During stimulation of the sinus nerve blood flow declines precipitously Decrease in flow is not maintained despite continued stimulation

but are not included in the graphs because measurement of such small changes was of limited accuracy

Constant stimulation for as long as five minutes in the lower frequency range produced sustained rises in blood pressure and heart rate At the higher levels of stimulation the rather large increases in blood pressure were not always maintained

When perfusion pressure equaled systemic pressure muscle blood flow was little changed as blood pressure rose during sinus nerve stimulation Thus the change in resistance in resting muscle essentially counterbalanced the blood pressure rise so that blood flow was constant (Fig 1) After stimulation of the sinus nerve was withdrawn there was a prompt and striking increase in muscle blood flow which appeared to be related to the intensity of the previous response The increased muscle blood flow often persisted for more than 60 seconds

When muscle perfusion pressure was maintained constant muscle blood flow decreased during sinus nerve stimulation indicating an increase in intravascular tension and resistance At the higher frequencies especially the decrease in muscle blood flow was not maintained and soon gave way to gradually increasing flow despite continued stimulation (Fig 6) When the stimulus was withdrawn there was a striking increase in muscle blood flow above the control level.

The effects of sinus nerve stimulation on the functions analyzed occurred promptly (Fig 1) After a lag no more than 3 to 5 seconds blood pressure heart rate and when perfusion pressure was held constant muscle blood flow

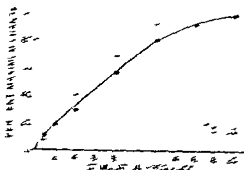
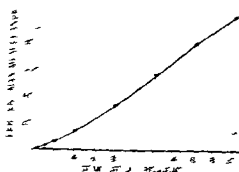




Fig. 2. [illegible] arterial pressure change during [illegible] stimulation. [illegible] standard deviation of mean.

Fig. 3. Change in heart rate during [illegible] stimulation.



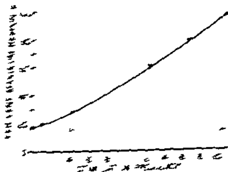


Fig. 4. Change in [illegible] vascular [illegible] with [illegible] pressure during [illegible] stimulation.

Fig. 5. Muscle [illegible] during [illegible] stimulation. [illegible] Control [illegible] [illegible] more than [illegible] resistance.

The stimulus response curves for change in [illegible] pressure, heart rate and resistance are [illegible] Fig. 2 [illegible] changes in [illegible] each stimulation frequency [illegible] the maximum change observed. In Fig. [illegible] resistance [illegible] absolute terms. The maximum resistance [illegible] with stimulation [illegible] average [illegible] after stimulation [illegible] the maximum.

Maximum responses [illegible] measured [illegible] cycles per second [illegible]

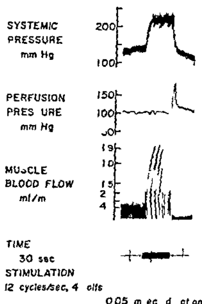


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The effects of sinus nerve stimulation on the functions analyzed occurred promptly (Fig 1) After a lag no more than 3 to 5 seconds blood pressure heart rate and when perfusion pressure was held constant muscle blood flow

changed in parallel until peak values were reached Maximum change usually developed within 30 seconds of continued stimulation When *sinus nerve* stimulation was stopped the parameters rather promptly returned toward the control level Delay in returning to the control value was not prolonged by increasing the frequency of stimulation

Discussion

The present study has demonstrated that in the cat anesthetized with chloralose bilateral stimulation of the sinus nerve with impulses of low voltage and short duration is associated with a rise in blood pressure an increase in heart rate and an increase in muscle vacular resistance directly proportional to the stimulation frequency Stimulation of the sinus nerve when other anesthetics are used causes a fall in blood pressure and a decrease in muscle vascular resistance due to baroreceptor inhibition of sympathetic vasoconstrictor discharge (FRUMIN *et al* 1953) and it is apparent that the anesthetic chloralose plays an important role in modifying the results obtained Species differences are also a factor for sinus nerve stimulation yields depressor responses in the chloralose anesthetized rabbit and dog (NEIL *et al* 1949)

NEIL *et al* (1949) showed that in the cat chloralose depressed baroreceptor function both centrally and peripherally but concluded that the central action was the major factor in producing the pressor response to sinus nerve stimulation There is other evidence that the action of chloralose on the central nervous system is one of selective depression Thus VON EULER and SODERBERG (1952) have shown that chloralose depresses the direct effect of CO_2 on the respiratory center but does not effect the respiratory response to injection of lobeline which acts by exciting the peripheral chemoceptors Although final interpretation must await more definitive information on the locus and mechanism of chloralose anesthesia the stimulus response curves obtained in the present work may represent an approximation of the chemoreceptor response which in other preparations is masked by predominance of baroreceptor function

Several workers have demonstrated an increase in vascular resistance during stimulation of the chemoceptors by hypoxia or chemical substances DALY and SCOTT (1962) have recently confirmed that muscle vascular resistance increases following hypoxic stimulation of the carotid bodies in the dog The rise in blood pressure and vascular resistance observed in the present experiments are similar to the effects following carotid body excitation by anoxia and lend weight to the interpretation that sinus nerve stimulation in the chloralose anesthetized cat excites primarily chemoceptor pathways

The frequency response curves for muscle vascular resistance obtained with sinus nerve stimulation are similar to those reported by FOLKOW (1952) who stimulated the sympathetic vasoconstrictor fibers to muscle directly Both

FOLKOW's and the present data show maximum responses at approximately 20 cycles per second. FOLKOW's curves however have a greater slope in the lower frequency range. He found that at a frequency of 6—8 cycles per second already the constrictor response was 80 % or more of maximum. With stimulation of the sinus nerve in the present experiments 80 % of maximum muscle constrictor response was not reached until an average frequency of 15 cycles per second. The same is also true for the blood pressure and heart rate changes.

Several factors may have influenced the frequency response curves in the present experiments. The sinus nerves in the cat are quite small and the initial handling required to set up the experiment may have inflicted some damage and reduced sensitivity. It is possible that a vasodilator may have been released. The animals were not adrenalectomized and an interaction between a systemic vasodilator and the constrictor response to sinus nerve stimulation may have occurred. It should be pointed out, however, that the curves derived from direct stimulation of the lumbar sympathetics represent selected data. From a large number of experiments a small group of preparations showing the most active vasoconstriction were chosen to plot the curve. The present data is entirely unselected and represents all the responses obtained.

The increase in muscle blood flow following cessation of sinus nerve stimulation despite maintenance of blood pressure at control levels (Fig. 1) suggests that a vasodilator accumulated during the stimulation. It is unlikely that this dilator was released from muscle since blood flow was maintained during the pressure rise and the limb remained at rest without muscle fasiculations. Muscle blood flow fell only in those experiments in which perfusion pressure to muscle was artificially held constant during the sinus nerve stimulation. Under this special circumstance locally accumulating vasodilator undoubtedly was a factor in the inability of the muscle to maintain its vasoconstriction.

The effect of chemoceptor stimulation on heart rate is variable. In the dog under controlled ventilation the response is cardioinhibitory and may be reversed by atropine or section of the cervical vagosympathetic trunks. In the dog with spontaneous ventilation stimulation of the carotid bodies causes acceleration (DALY and SCOTT 1958, 1962). In the cat NEIL (1956) was unable to demonstrate any change in the tachycardia of systemic anoxia despite varying oxygen tension in the carotid body perfusate. Stimulation of the carotid body directly with cyanide caused an increase in heart rate (LANDGREN and NEIL 1952).

The increase in heart rate observed during sinus nerve stimulation in the present experiment may reflect both a direct neurogenic effect and catechol discharge from the adrenal medulla. The response occurred within seconds of application of stimulus. Immediately after the stimulation was withdrawn the rate returned to the control level. A maximum response at 16—20 cycles is similar to that obtained by stimulating the thoracic sympathetic to the heart directly (FOLKOW *et al.* 1956). The increase in heart rate is of interest in view

of the observations of NEIL (1956) but is in accord with the experience of DALY and SCOTT (1958 1962) when they divided the vagosympathetic trunk.

The maximum frequency of response observed in the present study is in accord with the data of VON EULER LILJESTRAND and ZOTTERMAN (1940) who studied the chemoceptor fibers directly They estimated that the maximum frequency of discharge for a single fiber to be not greater than 10 per second under physiological conditions (16 4 % O_2 and 4 6 % CO_2 in the inspired air) Injections of lobeline, almost certainly providing a supramaximal stimulus gave rise to frequencies as high as 30 per second That the chemoreceptor fibers are limited in their discharge frequency is reasonable in view of their small size less than 5 micron in diameter (DECASTRO 1951)

Frequency response data from the chemoceptor fibers of the sinus nerve when correlated with similar data from the sympathetic vasoconstrictor fibers provide indirect information on the function of the central nervous system Pressor reflexes are mediated through the sympathetic vasoconstrictor fibers (BERNTHAL *et al.* 1945) and depend upon the integrity of the medulla oblongata (ALEXANDER 1946) The question may be posed whether the vasoconstrictor center in the medulla functions as a relay station or amplifies or reduces the frequency of afferent stimuli The similarity between the stimulus response curves for resistance change in muscle derived from direct sympathetic vasoconstrictor stimulation and those obtained from stimulation of the sinus nerve in the chloralose anesthetized cat suggests that the vasoconstrictor center provides a 1 1 relay between inflow of afferent stimuli from the receptor areas and outflow to the sympathetic fibers In this regard it is of interest that PITTS *et al* (1941) found a linear relationship between the frequency of hypothalamic stimulation and cervical sympathetic discharge in a ratio of 25 1 and suggested that the medulla was the site at which the frequency of the sympathetic fibers was limited (PITTS and BRONK 1942)

This work was performed during a Special Research Fellowship from the U S National Heart Institute in the laboratory of Professor Bo j U nas, Department of Pharmacology Karolinska Institutet, Stockholm. Laboratory expenses were defrayed by National Heart Institute Grant H-6633 to Professor U nas.

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In a previous paper a method was described for correlative studies of EEG and blood brain barrier phenomena under conditions facilitating separate effects on either one or the other parameter (FLODMARK and STEINWALL 1962) Thus it was possible to induce alteration of barrier functions without concomitant influence on the neuronal activity as reflected in the EEG The idea was that suitable noxious agents applied from inside the cerebral vessels via the carotid artery under special control measures could exert their effect primarily on barrier structures interposed between blood and neurons Theoretically if a barrier lesion is slight and reversible no significant EEG changes will occur On the other hand persistent barrier dysfunction would be expected to produce progressive disturbances of the metabolic milieu of the neurons and eventually interfere with their electrical activity

The last mentioned alternative is analyzed in the present study the aim of which has been to bring about a persistent barrier lesion and follow its influence on the EEG As a barrier damaging agent mercuric dichloride was chosen According to STEINWALL (1961) the mercuric ion even in very low concentrations injures the blood brain barrier presumably by a poorly reversible action on tissue components essential for the metabolic maintenance of barrier mechanisms The barrier function studied is that hindering the passage from the blood into the brain of various organic acids including acid dyes

Methods

The e perim ntal proc dures re fully described n a pr ous paper (FLODMARK and STEINWALL 1962) and will only be um marily outlined h re Adult rabb ts in urethan anesthesia were used EEG as ontinuou ly ecorded by means of ntraosseally applied s l er or plat num plated electrodes conne ted to an 8-channel t an i tor ized Kai er EEG machin Th me curic d hlor de olut ons n concent ations of 0 01—0 04 mM were applied thin th e els of on h m sphe for about half a minute a the p lat ral nt rnal carot d arter with xpul on of th blood dur ng the i je t on visually controll d through a t ephin open g Th mer ur ls r freshly prep red as app ox matel i ot nic and n utral solut ons n ph s ological saline

The barr r function as tested during th run of th exp ment by ntra er ous administr tion of u tabl o gan ds no m lly unable to pass f om blood to b ain but found to au e EEG hanges aft in asion th ough a defecti e barrier s GONSETTE (1956) FLODMARK a d STE W ALL (1962) Fo such nd cation penic ll n G U okon (sod um ac t o t or bromcresol green e d Th doses (per kg body weight) we e of th ord r 0 01—0 05 g p n ill n 3—4 g Urokon 0 1—0 2 g bromcr ol gre n

In order to nd ate th x te i e and xt nt of ba r e damag on in pection of the removed b ain trypan bl e or ome oth c d d e as dmi i st ed intra nou ly in all xperim nts us ally a few minutes b f e th t rm nation The d gre of staining of the damaged egion as ougl ly est mated as sl ght + medium ++ o l avy +++

The du ation of the expe ment ft th arot d njection varied betw een 30 and 5 hours

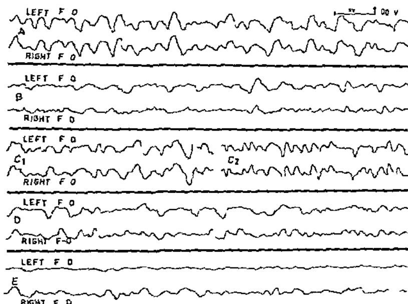


Fig 1 (no 3 in Table I) *EEG urse ft r l ft ided arotid inj tion f 0 03 mM HgCl a th pon taneous pass ge through the stag s I—III*

A Control r co d (urethan an sthesia)
B Stage I ju t after the end of the carotid injection low wa es on the l ft (injected) de arousal p ttern on the ght side.
C Stage II EEG symmetry regained. C1 3 min after HgCl injection (medium sleep) C2 about 1 hour later (light sl p)
D E Stag III Progressive EEG d ter o atı n on the left s 1 D after bout 2 hours E t the end of th experiment (about 1 hour after D)
(F—O b polar fronto-occ pital leads)

Results

Effects on the blood brain barrier according to the dye test The data concerning the carotid injection of mercuric dichloride and the results of the dye test are summarized in Table I together with a schematic denotation of the EEG course The blood brain barrier damage was indicated as extravasation of the dye in the left hemisphere which had been perfused with the mercuric solutions Three rabbits showing neither staining nor EEG changes have been excluded In a few animals the narrow medial strip of the right hemisphere nourished by the common anterior cerebral trunk was also stained however this restricted involvement of the control hemisphere did not interfere principally with the EEG observations during the experiments

The left hemisphere showed sometimes macroscopic swelling at autopsy It was also observed that intravenous administration of hypertonic glucose or urea could exert an anti-oedematous effect as judged by transient EEG improvement. This aspect however was not systematically analyzed

Table I Data concerning the carotidally injected mercurial, the barrier damage indicator and the EEG course

Exp no.	Injected HgCl			Indicator dye	Left sided extravasation of dye	EEG course			Remarks
	Conc. (mM)	Amount (ml)	Application (sec)			Stage			
						I	II	III	
1	0 02	1	35	Trypan blue	+++	+	−	−	Spontaneous
2	0 04	7	27	Trypan blue	+++	+	−	−	EEG course through
3	0 03	20	0	Trypan blue	++	+	−	−	stages I III (see
4	0 03	8	30	Trypan blue	+	+	+	−	text) No cut at n
	0 015	16	35	Trypan blue	++	+	−	−	EEG ct ation f ter spontaneous passage into stage III
6	0 018	8	24	Trypan blue	++		+	+	EEG cu t on in stage II possibly
7	0 018	19	25	Trypan blue	++	−	−	+	interfering with
8	0 02	6	35	Bromcresol green	++	−	−	+	later EEG course
9	0 03	7	40	Bromcresol green	+++	−	−		EEG t at on in stage II persistent
10	0 03	11	45	Bromcresol green	+++	−	−		and obscuring the later EEG course
11	0 01	8	40	Bromcresol green	+	−	−		
12	0 01	8	35	Trypan blue	++	+	+		
13	0 018	8	30	Trypan blue	+	−	−		
14	0 04	19+6	17+15	Trypan blue	+	+	−		
15	0 02	8	30	Trypan blue	(+)	−	−	—	Experiments
16	0 016	17	30	Trypan blue	+	−	−	—	terminated within
17	0 02	8	30	Prontosil solubil	+	−	−	—	stage II

Effects on the EEG Before the carotid injection control records with practically symmetrical patterns and arousal responses to pain stimuli were obtained Small differences in amplitude were tolerated if the frequency patterns were similar on the right and left sides

During the course of most experiments 3 stages could be recognized in the EEG

Stage I which occurred in connection with the carotid injection started after 10—20 sec and was characterized by marked asymmetry owing to a decreased frequency and depressed amplitude on the injected (left) side The pattern was principally the same as that seen on injection of physiological saline (FLODMARK and STEINWALL 1962) although lasting somewhat longer (up to 5 min) in some of the present experiments

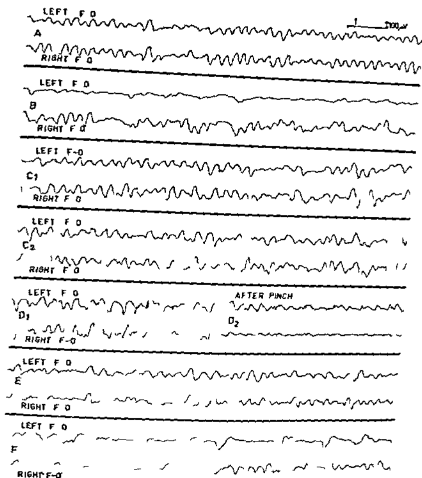


Fig 2 (no / in Table I) *EEG change in the barrier dam d (left) s de pr vol d during st g II Partial eve s on b fo the EEG dete iorat on n the t r al a e*

A Control ecord almost symmetrical pattern (sl ghtly low r a nplitudes on the left side)
B Stage I ma k d l ft s ded amplit de depress on during the carotid j ct n of HgCl
C Stage II pract cally symmetr cal p ttern g ned. (C1 5 min C2 27 min after th ca t d inj ction)
D Left ded sharp potentials 6—7 min after v administr tion of 0 03 g kg penicill n C (injected abo t 30 min fte HgCl pplication) D1 medium sl ep D2 ar usal aft pinch
E Two hours late E ident altho h not compl t reversal of the l ft-s ded EEG changes.
F Left ided EEG d terio ation in th terminal st g about 1 hour after E.
(F—O b polar fro o-occip tal leads

These EEG changes then typically reverted to an almo t symmetrical record essentially like the control Thus *stage II* could persist for more than two hours but in some experiments where the dye test indicated a pronounced barrier damage the period of symmetry was considerably shortened to as little as a few minutes

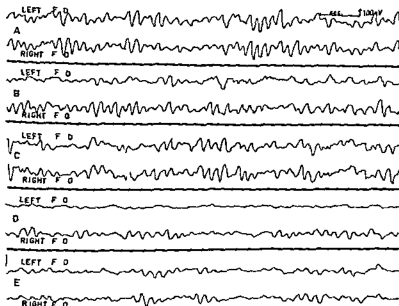


Fig 3 (no 16 in Tabl 1) *Indicat f barr e d) functi u th t e II by ans f t e us loadin u th od um ac t o te (L oko)*

A Contr l ecord.
B Stag I Asy nm try dur g th l ft s d d a otid inj ct n f HgCl
C Stag II Sy nm tr cal patt rn gained (3 min l te)
D 7 min after l ading th Ur ko in tag II ma ked ampl tude dep es n nly on th left d
E 1 min lat left-s d d EEG es nt lly st r d (Th p riment as t rmin ted 3 min l et ab t 30 min after th carot d inject on)
(F—O b polar f nto c p tal leads)

Stage III was chara terized by graduall) increasing EEG abnormalities on the affected side leading to marked asymmetry Terminally the left side record was often totally flattened

The spontaneous passage through the three EEG stages illustrated in Fig 1 was observed in 5 animals (nos 1—5 Table I) In 4 of these no measures interfering with the EEG were undertaken after the carotid injection until the application of the dye test in stage III In the fifth experiment where Urokon was injected long after the passage into stage III it was observed that even in this stage the electrical activity could be reactivated as seen by the ap pearance of series of spikes on the left side

The control of the barrier state during the symmetrical stage II by means of intravenous loading with acid compounds was performed in 9 experiments (nos 6—14) In all instances the administration of penicillin G Urokon o bromcresol green revealed a defective barrier in the left hemisphere in w the invading acid e oked s gnificant disturbances in the EEG within 1

The abnormalities consisted of decreased frequency amplitude depression and often intermittent sharp potentials In 3 experiments (nos 6—8) these activating effects showed an obvious regression and presumably did not interfere with the subsequent development of the terminal stage III These events are illustrated in Fig 2 In 6 instances (nos 9—14) the evoked changes persisted until the end of the experiment thus obscuring the spontaneous EEG course In 3 animals (nos 15—17) the dysfunction of the barrier in stage II was indicated as left sided staining when the dye was administered in this stage and the experiments terminated already before the appearance of the gross abnormalities characterizing the terminal stage In exp 16 the left sided barrier defect was additionally demonstrated by means of a reversible EEG influence after intravenous loading with Urokon (see Fig 3)

Comment

On analysis of the results obtained we are aware of that the EEG is but a summary indicator of the metabolic state of the neurons and that the barrier phenomena studied represent only a limited aspect of the intricate machinery which regulates the blood brain exchange of matter Nevertheless the interrelations between the two parameters as they were observed in the present investigation offer a basis for some interpretations which in the following will be delineated in regard to the 3 stages in the EEG course

The asymmetrical EEG pattern in stage I appearing during the carotid injection can for the most part be attributed to the anoxia at the blood expulsion as previously discussed (FLODMARK and STEINWALL 1962) The tendency toward prolongation of the effect as compared to that seen on injection of physiological saline might reflect a transient circulatory insufficiency due to local vascular reactions to the mercuric solutions

The almost complete reversion to symmetry in the EEG in stage II and the often long duration of this stage are the most important findings The EEG normalization after the acute influences at the carotid injection indicates that the action of the mercurial did not imply any appreciable impairment of the circulation and oxygen supply during stage II On the other hand it is evident that there was a dysfunction of the barrier in this EEG stage as demonstrated by the unilateral effects exerted by abnormally penetrating organic acid compounds It is tempting to assume that the mercuric ions acted primarily within barrier structures interposed between the blood and the neurons (cf DEMIS 1953) The strong affinity of heavy metal ions to certain chemical radicals in the tissue implies a restricted mobility as well as a poor reversibility of the functional alterations evoked These circumstances favour the production of a lesion primarily restricted to the barrier for a long period as seems to be the case in stage II

In the last stage (III) the deteriorating electrical activity on the damaged side might be the effect of a direct attack on the neurons from slowly migrating

mercuric ions According to the considerations above however it seems more plausible to assume that the alteration of the EEG reflects in this instance the increasing and complex metabolic disturbances arising from the barrier dysfunction *per se*

Further experiments are now in progress for investigation of the effect of agents whose influence on the blood brain barrier seems to be reversible and based on other mechanisms of action than the mercuric lesion

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FLO MARK, S and O STEINWALL A m th od f tud f th int rrelat on bet en EEG and blood b ain barrier phenom n *4 ta phy iol c nd* 1962 *56* 112—119

STEINWALL, O T ansport mechanism. in c rtain blood b ain b rrie ph n m na — a hypoth esis *lct p y h l* (Kbh) 1961 *36* S ppl 150 314— 18

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In the last stage (III) the deteriorating electrical activity on the damaged side might be the effect of a direct attack on the neurons from slowly migrating

pyruvate transaminase-deamidase activity in rat liver homogenates (MEISTER and DOWNEY 1956 COPENHAVER *et al* 1959) Aspartic acid glutamic acid and valine glutamic acid transaminase activites are decreased by isoniazid in *Escherichia coli* extracts (HICKS 1959) and a decrease in glutamic-oxaloacetic transaminase was also found in the whole blood of patients receiving isoniazid (SASS and MURPHY 1958) In many investigations hydrazides have had no effect on transaminases *in vivo* or *in vitro* Activation of transaminases has been found however by some authors (GONNARD and NGUYEN PHILIPPON 1959 BONAVITA and SCARDI 1959) in experiments *in vitro* Very little is known of the possible activation of transaminases by hydrazides *in vivo* (PLETSCHER *et al* 1960)

In preliminary experiments we have found that alanine transaminase activity in livers of developing chick embryos was markedly increased after administration of nialamide or isocarboxazid Lactate dehydrogenase was also slightly affected These results prompted us to study further the effect of various types of hydrazides and deoxypyridoxine on liver alanine and aspartate transaminases and on lactate dehydrogenase in chick embryos The liver keto acid content has also been determined in some experiments

Material and Methods

The xperimental material emplo ed was fertil ed eggs White Leghorn obtained from a commerc al hat hery Sa no n n O Th eggs ere incubated at 37 5 ± 0.5 C.

The follo ing compounds used in th study Semicarbazide h drochloride (E. Mer k, Darmstadt am noguan d n ulph t The British Drug Houses, Poole) isoniaz d Fluka A G St Gallen p on az d phosphat Mars ltd F Hoffmann La Roche & Co Bas l iso a bo az d Marplan F Hoffmann La Roche & Co Basel) nialamid (N amid Chaz Pf er Co In New Y k and 4-d oxypyridoxine hyd ochlorid Fluka A G St G llen Th y w dminist ed in 0 9 per c nt sodium chloride to th horioallan o m mb an of hi k embryo at 14th—1 th da of development The ont l t ated w th a equal amount f 0 9 per cent sod um chlo d All mbryo on xpe m n al se es k lled at th sam t m

After th kill ng of th mbryo h l mo ed w gh d and mmed ately homog nized n 10 lum s pe ght t c ld 0 9 pe nt sod um hloride olut on w th a Potter El ehj m t pe h m g z f h l mog nates re to ed at — 0 C until an l z d

Th nz m t er d m n l b th f ll ng m thods Al n transaminase (EC C l f rm l known as gl tam p ru t ansam na was d te m n d a co d ng to WRO E SK d CA A D 1 and th esults wer ont oll d us ng th n m m thod of WROB k and LADUE 1 5 Aspartat t an am nas (EC 6 1 1 f rm l k n as gl tam c-oxaloa ti transaminase was d t mined a o d ng to D 19 and th esults w ont olled us ng an nz m m thod (SGOT Test B ochem a Loeh ng M n h m La tat d h d ogenase (EC 1 1 1 27 as d term ned b th m thod f WROBLE SKI and LADUE 19 5)

Repo of th Commiss Enz mes f I I rna nal C ion f B ochemis ry Per P ess, Oxfo d 1961

Table I. Effect of nialamide, isocarboxazid and deoxypyridoxine on liver alanine and aspartate transaminases and lactate dehydrogenase in chick embryos

The enzyme activities are expressed as units per mg of tissue wet weight (mean ± S.D.). Number of embryos in parentheses. Dosage per embryo: Nialamide 15 μmoles, isocarboxazid 3 μmoles, deoxypyridoxine 15 μmoles. The embryos were killed at the 17th day of development. For the determinations livers of two embryos were pooled.

Treatment	Administration time before killing (hours)	Alanine transaminase		Aspartate transaminase		Lactate dehydrogenase	
		U/mg	Change	U/mg	Change	U/mg	Change
Controls (12)	40	22±3 4	—	1 8±3 4	—	643±51	—
Nialamide (10)	10	39 5±9.3	+ 23	114±7 3	−11	541±37	−17
Nialamide (12)	40	91 4±11	+180	92±9 6	−28	596±34	− 8
Isocarboxazid (10)	10	43 6±2 7	+ 35	125±3 4	− 2	651±29	± 0
Isocarboxazid (10)	40	73 4±11	+130	114+9 3	−11	647±8	± 0
Deoxypyridoxine (6)	40	33 7±7 6	+ 5	117±5 3	− 9	616±80	− 5
Deoxypyridoxine (6)	40 and 10	44 9±8 6	+ 39	110±0 7	−14	568±49	−12

$P < 0.05$ $P < 0.01$ $P < 0.001$

Enzyme activities are presented as units per mg of liver tissue (wet weight). The units are defined as follows. One unit of alanine or aspartate transaminase is defined as the amount of activity that results in the formation of 1 μg of pyruvate in the assay system (WROBLEWSKI and CABAUD 1957, DUBACH 1957). One unit of lactate dehydrogenase is defined as the amount of activity that gives a decrease of 0.001 per minute in the extinction at 340 mμ (WRÓBLEWSKI and LADUE 1955).

The keto acids were determined according to DE SCHEPPER *et al.* (1958). α-ketoglutaric acid and pyruvic acid were separated by paper chromatography.

The statistical treatment of the results has been made using Student's t test.

Results

Effect of Hydrazides and Deoxypyridoxine on Liver Transaminases and Lactate Dehydrogenase in Chick Embryos. — The effect of nialamide, isocarboxazid and deoxypyridoxine on liver transaminases and lactate dehydrogenase in chick embryos is presented in Table I. In these experiments the dose of nialamide was 15 μmoles/embryo given as a single injection, deoxypyridoxine was given as one or two doses of 15 μmoles/embryo each time. The dosage of isocarboxazid was much smaller (3 μmoles/embryo) owing to its poor solubility.

Aspartate transaminase activity was significantly decreased after administration of all these drugs, whereas alanine transaminase activity was on the contrary significantly increased. Both these effects increased with time elapsed after administration. Nialamide and deoxypyridoxine decreased slightly liver

Table II Effect of various hydrazides and deoxypyridoxine on liver alanine and aspartate transaminases and lactate dehydrogenase in chick embryos

The enzyme activities are expressed as units per mg of tissue wet weight (mean ± S D Number of embryos in parentheses. Dosage 5 μmoles per embryo except isocarboxazid 3 μmoles. The embryos were killed at the 18th day of development

Treatment	Administ time before killing (hours)	Alanine transaminase		Aspartate transaminase		Lactate dehydrogenase	
		U/mg	Change	U/mg	Change	U/mg	Change
Controls (7)	40	41.3±5.8	—	137±13	—	612±66	—
Semicarbazide (7)	40	44.5±5.0	+8	139±11	+1	659±92	+8
Aminoguanidine (7)	40	67.7±13	+64	132±11	−4	633±45	+3
Nialamide (6)	40	80.8±23	+95	123±7.7	−10	586±66	−4
Isocarboxazid (6)	40	9 4±17	+130	117±11	−15	662±72	+8
Iproniazid (6)	40	93.1±20	+125	122±7.1	−11	6 5±58	+7
Isoniazid (6)	40	120±14	+190	101±5.5	−26	640±6	+5
Deoxypyridoxine (6)	40 and 10	77.2±20	+87	117±20	−1	606±81	−1

P < 0.0 P < 0.01 P < 0.001

lactate dehydrogenase activity isocarboxazid had no effect on lactate dehydrogenase at the dose level used

The effect of various types of hydrazides and deoxypyridoxine is presented in Table II In these experiments the dose was cut down to 5 μmoles per embryo for all hydrazides except isocarboxazid (3 μmoles/embryo) Deoxypyridoxine was given twice 5 μmoles/embryo each time. Semicarbazide had no effect on liver transaminases activities Aminoguanidine nialamide isocarboxazid iproniazid isoniazid and deoxypyridoxine clearly increased the alanine transaminase activity and decreased the aspartate transaminase activity Isoniazid was the most effective of these drugs At this dose level there were no consistent changes in liver lactate dehydrogenase activity

To study the effect of hydrazides as a function of time elapsed after their administration isoniazid and nialamide (5 μmoles/embryo) were given at different intervals before the determination of transaminase activities The results are seen in Fig 1 The increase in alanine transaminase activity is more rapid after nialamide than after isoniazid On the contrary the decrease in aspartate transaminase is more rapid after isoniazid

Effect of Nialamide Isocarboxazid and Deoxypyridoxine on the Liver Keto Acid Content in Chick Embryos — The results are presented in Table III The determination of keto acids was made from the same livers from which the enzyme activities are presented in Table I Only negligible amounts of other keto

Table I. Effect of nialamide, isocarboxazid and deoxypyridoxine on liver alanine and aspartate transaminases and lactate dehydrogenase in chick embryos

The enzyme activities are expressed as units per mg of tissue wet weight (mean ± S.D.; number of embryos in parentheses). Dosage per embryo: Nialamid 1[illegible] µmoles, isocarboxazid 3 µmoles, deoxypyridoxine 1[illegible] µmoles. The embryos were killed at the 1[illegible]th day of development. For the determinations livers of two embryos were pooled.

Treatment	Administration before killing (hours)	Alanine transaminase U/mg	Change %	Aspartate transaminase U/mg	Change %	Lactate dehydrogenase U/mg	Change %
Controls (1[illegible])	40	[illegible]2±3.4	—	1[illegible]8±3 4	—	6[illegible]9±[illegible]1	—
Nialamide (10)	10	9[illegible]±[illegible].3	+ 2[illegible]	114±7[illegible]	−11	[illegible]1±37	−1
Nialamide (12)	[illegible]0	[illegible]1 4±11	+1[illegible]0	[illegible] ±9[illegible]	−[illegible]8	[illegible] ±34	− 8
Isocarboxazid (10)	10	4 [illegible]±2[illegible]	+ [illegible]	1[illegible] ±[illegible] 4	− 2	6[illegible]1±[illegible]9	± 0
Isocarboxazid (10)	4[illegible]	34±11	−1[illegible]0	114+9.3	−11	6[illegible]±8[illegible]	± 0
Deoxypyridoxine (6)	4[illegible]	[illegible]3[illegible]±7 6	− [illegible]	11 ±[illegible] 3	− 9	616±[illegible]0	− [illegible]
Deoxypyridoxine ([illegible])	40 and 10	4[illegible]9±8[illegible]	− [illegible]	110±0 7	−14	[illegible]68±49	1[illegible]

P < 0.0 P < 0.01 P < 0.001

Enzyme activities are presented as units per mg of liver tissue (wet weight). The units are defined as follows. One unit of alanine or aspartate transaminase is defined as the amount of activity that results in the formation of 1 µg of pyruvate in the assay system (WRÓBLEWSKI and CABAUD 1957; DEBACH 1957). One unit of lactate dehydrogenase is defined as the amount of activity that gives a decrease of 0.001 per minute in the extinction at [illegible]0 mµ (WRÓBLEWSKI and LADUE 19[illegible]).

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Aspartate transaminase activity was significantly decreased after administration of all these drugs, whereas alanine transaminase activity was on the contrary significantly increased. Both these effects increased with time elapsed after administration. Nialamide and deoxypyridoxine decreased slightly liver

Effect of Nialamide on Liver Alanine and Aspartate Transaminases in Rat. — To check the effect of nialamide on liver transaminases in the rat two series of experiments were made. In the first series rats were injected intraperitoneally with nialamide (70 μmoles/kg) daily for two weeks, in the second series one single injection (700 μmoles/kg) of nialamide was given per rat. The animals were killed 24 hours after the last injection and the liver transaminase activities were determined. No change, however, was seen in the liver transaminase activities in the experimental groups when compared with controls.

Discussion

The mechanism of the inhibition of pyridoxal phosphate dependent enzymes by hydrazides, especially isoniazid, has been widely discussed by many authors. It has been suggested that the inhibition by isoniazid is due to competition between pyridoxal and isoniazid, for there is a structural resemblance between these two substances and the inhibition is reversed in many cases by pyridoxal (YONEDA *et al.* 1952, YONEDA and ASANO 1953, BOONE and WOODWARD 1953). VITTER *et al.* (1954) and DAVISON (1956) have suggested that the inhibition of these enzymes by hydrazine derivatives is due to the formation of hydrazonic compounds with pyridoxal. According to DAVISON (1956) a free hydrazine group is needed for inhibition *in vitro*, because iproniazid with a substituted terminal amino group was not effective. *In vivo*, however, in rats iproniazid was effective and this was apparently due to its conversion to free hydrazine.

In the present experiments with developing chick embryos various hydrazine derivatives decreased liver aspartate transaminase activity, whereas a marked increase was observed in liver alanine transaminase activity. These effects lasted at least 70 hours in embryos treated with nialamide or isoniazid.

Isoniazid with a free hydrazine group had the greatest effect in these experiments. When the effects of isoniazid and nialamide, which has a bound hydrazine group, are compared, it can be seen (Fig. 1) that isoniazid decreased more rapidly the activity of liver aspartate transaminase, whereas the increase in alanine transaminase was more rapid after nialamide.

Liver lactate dehydrogenase activity was only slightly decreased by large doses of hydrazides. GUTMANN *et al.* (1954) have reported similar results using purified mammalian lactate dehydrogenase and isoniazid. Some increase in liver keto acid content was also found in the present experiments, but the effect was transitory. This is in agreement with the results of GEY and PLETSCHER (1961), who found an increase in the pyruvic and lactic acid content of rat blood after treatment with iproniazid.

In the experiments of ROSEN (1959) with B_6 vitamin deficient rats both a small decrease in liver aspartate transaminase and a small increase in liver alanine transaminase can be seen after treatment with isoniazid and iproniazid. In the present work similar but much more pronounced changes were consistently

noted in the livers of developing chick embryo after administration of hydrazides. It is remarkable that the pyridoxine antimetabolite deoxypyridoxine has the same activating effect on liver alanine transaminase in chick embryos as hydrazides. The inhibitory effect of deoxypyridoxine on aspartate transaminase has been noted also in mouse liver by SHAPIRO *et al.* (19[illegible]3).

In our experiments the liver transaminases were not affected by malamide in normal rats. It seems possible that developing embryonal tissues are more liable to the effects of hydrazides and B_6 antagonists than adult tissues. Further work is needed to clarify the mechanism of the contrasting effects on alanine and aspartate transaminases of hydrazides and deoxypyridoxine.

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Effect of Thyroxine on the Hydroxyproline in Rat Urine and Skin

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Abstract

KIVIRIKKO K I M KOIVUSALO O LAITINEN and M LIESMAA. *Effect f thyroxine on the hydroxyprol ne in rat urine and skin* Acta phys ol scand 1963 57 462—467 — The effect of L-thyroxine on the metabolism of collagen in rats was studied by analyzing the urine for free and total hydroxyproline In addition the hydroxyproline of the neutral salt and citrate soluble fractions of the skin was determined Both free and total hydroxyproline were equally increased in the urine after the administration of L-thyroxine The increase was of the same magnitude in two four and twel e months old rats although the basal excretion alues of hydroxyproline were significantly lower in the older rats The content of hydroxyproline in both neutral salt and citrate soluble fractions of the skin was slightly decreased after the admin stration of L-thyroxine

Thyroid hormone has an inhibitory effect on the connective tissue (ASBOE HANSEN 1958) Most investigations on the mechanism of this effect indicate changes in the metabolism of sulphomucopolysaccharides (MOLTKE 1958 WHITEHOUSE and BOSTROM 1961) whereas less is known of the possible changes in the metabolism of collagen

Hydroxyproline is a specific amino acid for collagen Therefore changes in hydroxyproline concentration have been used as an indicator of changes in the metabolism of collagen In the present work the effect of L-thyroxine on the urinary excretion of free and total hydroxyproline has been studied in rats In addition the hydroxypr line content of the soluble fractions of skin was determined

It is known that the urinary excretion of hydroxyproline (ZIFF et al 1956 KIVIRIKKO and LIESMAA 1958) and the tissue concentrations of ultrafiltrable

hydroxyproline (KOBRLE and CHVAPIL 1962) and soluble collagen (OREKHOVICH 1953, WIRTSCHAFTER and BENTLEY 1962) decrease with age. In addition hormonal effects on the collagen metabolism may be dependent on the age of the experimental animals (SETHI *et al.* 1961). Therefore it seemed worthwhile to compare the effects of thyroxine on the urinary hydroxyproline in young and old rats.

Material and Methods

The experimental animals were albino rats of Wistar strain. In preliminary experiments 4 months old female rats were used, the later series were made with 2, 4 and 12 months old male rats. The animals were weighed at the beginning of the experiments and after the urine collections. They were fed *ad lib.* with a commercial pelleted diet (Hankkija Oy) fortified with cheese and they were always allowed free access to water.

L-Thyroxine sodium (Orion Oy) was injected subcutaneously suspended in 0.9 per cent sodium chloride. The suspensions contained the desired amount in 0.1 ml and were made freshly daily. In the preliminary experiments thyroid extract (Thyranon, Organon) was injected subcutaneously in amounts corresponding to 10 μg of organically bound iodine. In all experiments controls were injected with 0.1 ml of 0.9 per cent sodium chloride.

Urine collections were made during a 14 hour period, the urine samples were stored at + 2 under toluene. During the 14 hour urine collection period only water was given to the animals. In the calculation of results the values were converted to μg per 24 hours. An aliquot was taken for the determination of free hydroxyproline and another aliquot was hydrolyzed with an equal amount of 12 N HCl for 3 hours at 124 C for the determination of total urinary hydroxyproline after neutralization with potassium hydroxide.

The neutral salt and citrate soluble fractions of skin were prepared as follows for the determination of hydroxyproline. After 9 daily injections of 50 or 100 μg of L-thyroxine, the animals were killed. A piece of the dorsal skin was excised immediately after killing and was freed from hair and fascia on an ice-cooled metal tray. The piece of skin was then homogenized in three volumes per weight of cold 0.45 M NaCl with Ultra Turrax homogenizer (Janke Kunkel). The amount of 0.45 M NaCl was increased from the 2 ml per g of tissue used by GROSS (1958) to 3 ml per g of tissue because this made the homogenization easier with the Ultra Turrax homogenizer. The extraction was continued for 24 hours with occasional stirring, whereafter the homogenate was centrifuged in the Spinco model L preparative ultracentrifuge at 60 000 × g for 30 min. From the clear supernatant 1 ml was taken for the determination of neutral salt soluble hydroxyproline. The residue after neutral salt extraction was suspended in two volumes of 0.2 M sodium citrate, pH 3.6, for 24 hours. After centrifugation at 60 000 × g for 30 min 1 ml of the supernatant was taken for the determination of citrate soluble hydroxyproline. The aliquots from the neutral salt and citrate soluble fractions were hydrolyzed with an equal amount of 12 N HCl at 138 C for 6 hours. After neutralization with sodium hydroxide hydroxyproline was determined from the samples. Since the water content of the skin was not determined, the hydroxyproline values obtained per 1 ml of neutral salt extract could not be exactly converted to values per g of tissue (wet weight). The conversion of analysis values to μg per g was made by multiplying the value for 1 ml neutral salt extract by 4 and the value for 1 ml citrate soluble extract by 2. This calculation gives about 10 per cent too high values for neutral salt soluble hydroxyproline, but the error is assumed to be the same in all samples.

Table I Urinary excretion of free and total hydroxyproline in 2 month-old rats The hydroxyproline values are expressed as μg per 24 hours (mean ± S D) the body weights in grams (mean ± S D)

Group		Weight	Free hydroxyproline	Total hydroxyproline
Controls (7)	0	149 ± 11	64 4 ± 29 6	511 ± 1 8
	I	160 ± 13	65 6 ± 21 7	540 ± 161
	II	167 ± 14	62 6 ± 22 4	558 ± 117
Thyroxine 50 μg daily (7)	0	150 ± 10	51 3 ± 14 0	390 ± 8
	I	158 ± 12	98 4 ± 47 5	[illegible]79 ± 169
	II	163 ± 10	109 7 ± 43 2	840 ± 189
Thyroxin 100 μg daily (7)	0	148 ± 9	63 [illegible] ± 9.2	474 ± 6[illegible]
	I	153 ± 12	93 [illegible] ± 26 9	[illegible]8 1 ± 196
	II	154 ± 14	118.2 ± 66 4	917 ± [illegible]16

$P < 0.05$

Number of rats within brackets.

0 = initial value I – after 5 injections II – after 8 injections.

Analytical methods Urinary hydroxyproline was determined in the preliminary experiments by the method of WISS (1949) and in the other series by the method of PROCKOP and UDENFRIEND (1960) Urinary proline was determined in the preliminary experiments by the method of TROLL and LINDSLEY (1955) Hydroxyproline in the soluble fractions of the skin was determined by a slight modification of the method proposed by KIVIRIKKO and LIESMAA (1959)

Statistical analysis of the results was carried out using Wilcoxon s S test (WALKER and LEV 1953) for the urinary hydroxyproline values and Student s t test for the skin hydroxyproline values

Results

In a preliminary series thyroid extract (Thyranon Organon) was injected daily into 10 female rats in amounts corresponding to 10 μg of organically bound iodine After 10 days treatment an increase of 114 per cent was found in the free hydroxyproline content of urine but the proline content in the urine increased only 29 per cent These results prompted us to further study on the effect of thyroxine on the hydroxyproline

The effect of daily injections of 50 and 100 μg of L-thyroxine on the urinary free and total hydroxyproline excretion in 2 months old male rats is seen from Table I These amounts of L thyroxine were found to retard slightly the weight gain of the rats The excretion of both free and total hydroxyproline was increased already after 5 injections and after 8 injections the values were somewhat higher still Standard deviations were rather high in all groups This is, however partly due to individual differences in the basal excretion of hydroxyproline For example in the control group the range of free hydroxyproline

Table II Urina y ex retion of f ee and total hyd oxypr line in 4 and 12 months old rats The hydroxyproline values are xp ess d as μg per 24 hours (mean ± S D) the body weights in grams (mean ± S D)

Ag (months)	Group		Weight	Free hydroxyproline	Total hydroxyproline
4	Contr ls (7)	II	?10 ± 19	40.. ± 10 6	34 ± 7b
	Thyr xine 20 μg daily (7)	II	202 ± 16	49 6 ± 16 8	393 ± 84
	Thyroxine 50 μg daily (7)	II	197 ± ?1	/6 0 ± 33 7	560 ± 133
1	Contr ls (7)	0	279 ± 26	32 1 ± 9 3	284 ± 55
		II	286 ± 32	29 1 ± 9 9	2?3 ± 39
	Thyroxine 50 μg daily ( )	0	280 ± 25	5 6 ± 7 6	58 ± ?/
		II	262 ± 24	35 7 ± 13 9	364 ± 56

P < 0 0?   P < 0 01

Number of r ts w thin b cket

0 = nitial   II = after 8 njections.

Table III The amount of hydroxypr line in 0 45 M NaCl olubl a d trate luble f a t ons f the sk n f 2 m th-old rats The values a e e pr s ed as μg per g wet wei ht f tis u (mean ± S D) In th tatist cal tr atment pooled values f the e perimental groups ar compar d with o t ol value

Group	NaCl-soluble hydroxyproline	Citrate soluble hydroxyproline
Controls	919 ± 138 (6)	335 ± 33 (7)
Thyroxine 50 μg d l	778 ± 11b (7)	294 ± 14 (7)
Thyroxine 100 μ da ly	7 9 ± 92 (6)	297 ± 35 (7)

P < 0 05   P < 0 01

N mber f ts w h b k t

excretion in the three urine collections was in one rat 32—42 μg and in another 66—87 μg and the total hydroxyproline values for the same rats had similarly different ranges (2 0—380 μg and 480—635 μg respectively) The administration of L-thyroxine increased however the excretion of hydroxyproline both in rats with high and low basal excretion The amount of free hydroxyproline was abo t 10—15 per cent of the total hydroxyproline in all groups and thus the administration of L-thyroxine did not change the ratio of free hydroxyproline to total hydroxyproline Evidently 50 μg of L-thyroxine was sufficient to give a maximum response because doubling of the dose to 100 μg did not further increase the excretion of hydroxyproline.

The values for 4 and 12 months old male rats are given in Table II. Although the basal excretion of both free and total hydroxyproline was significantly lower in these older groups than in the 2 months old rats no essential difference is seen in the effect of L-thyroxine.

A slight decrease in the content of neutral salt soluble and citrate soluble collagens was observed in the skin of 2 months old male rats after administration of L-thyroxine (Table III). The same animals had also been used for the urine collections recorded in Table I. Since there was no difference between the two doses of L-thyroxine the values for both groups receiving L-thyroxine were pooled for statistical treatment.

Discussion

In the present work, the urinary excretion of free and total hydroxyproline increased after the administration of thyroxine. The increase was of the same magnitude in all age groups although the basal excretion of both free and total hydroxyproline was significantly lower in the older age groups. This influence of age on the excretion of hydroxyproline is in agreement with earlier investigations (ZIFF et al. 1956, KIVIRIKKO and LIESMAA 1958, LINDSTEDT and PROCKOP 1961).

Since hydroxyproline is specific for collagen, urinary hydroxyproline has been regarded as a measure of collagen metabolism. LINDSTEDT and PROCKOP (1961) have shown that 3 different hydroxyproline pools exist in young rats suggesting the presence of rapidly metabolized forms of collagen. In later work PROCKOP (1962) studied the correlation of the specific activity of urinary hydroxyproline and the specific activity of the soluble and insoluble collagen of skin after injection of proline C^{14}. These results indicated that part of the soluble collagen is degarded rapidly to urinary hydroxyproline and some catabolism of insoluble collagen also occurs.

In the present work an attempt was made to correlate the changes in urinary hydroxyproline with the changes in the soluble collagen fractions of the skin. A slight decrease was found in the neutral salt soluble and citrate soluble collagens. In earlier investigations it has been shown that thyroidectomy or administration of thyroxine has no definite effect on the total hydroxyproline content of the aortic wall in normal rabbits (LORENZEN 1961, 1962). Neither was there any definite effect of thyroxine on the total hydroxyproline in mouse skin although the hydroxyproline content was significantly higher in the skin of thyrotrophin treated dwarf mice than in thyroxine treated dwarf mice (DYRBYE et al. 1959). Also in the experiments of GABAY et al. (1961) thyroxine had no effect on the total hydroxyproline content of bones in normal animals although thyroxine inhibited the increase in hydroxyproline of bones caused by methylene aminoacetonitrile. On the other hand the investigations of MOLTKE (1958) indicate that thyroxine has a definite inhibitory effect on the tensile strength of healing wounds.

The decrease in soluble collagens observed in the present experiments as co related with the increase in the urinary hydroxyproline suggests that thyroxin exerts its inhibitory effect on collagen by increasing the catabolism of solub collagen Because the changes in the soluble collagens however are rathe small and based only on wet weight determinations additional experiment are required to verify these results Further work is in progress to study th mechanism of the action of thyroxine on the metabolism of collagen

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Catecholamine Release and Uptake in Isolated Adrenergic Nerve Granules

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Abstract

EULER, U. S. v. and F. LISHAJKO. *Catecholamine release and uptake in isolated adrenergic nerve granules.* Acta physiol. scand. 1963. 57. 468—480. — Isolated storage granules from bovine splenic nerves incubated in neutral isotonic K phosphate give off noradrenaline at rates rapidly increasing with temperature. Noradrenaline in concentrations of 10—20 μg/ml in the incubation fluid inhibits or prevents the release. The noradrenaline thus retained is released at the normal rate after removal of the amine in the incubation fluid. After depletion the granules are able to take up noradrenaline and adrenaline to about the original content when exposed to catecholamines in concentrations of 10—20 μg/ml suggesting a limited storage capacity of the granules. It is suggested that the noradrenaline in the adrenergic nerve terminals is partly present in a free pool available for immediate release during nerve stimulation and that a lowering of the concentration in this pool causes the granules to give off the stored transmitter until an equilibrium is reached. The following resynthesis is assumed to continue until the free pool has reached a concentration preventing further release from the granules. This concentration is about the same as that normally found in bovine splenic nerves.

In previous communications (EULER and LISHAJKO 1961 a, b) it has been shown that intraaxonal granules isolated from bovine splenic nerves give off their noradrenaline on incubation in neutral isotonic K phosphate. The release rate is very low at temperatures below + 5° C but increases rapidly at higher temperatures. At 20° C the half decay time is about 50 min and at 37° C about 5 min.

Zum 60. Geburtstag von Herrn Professor A. von Muralt.

Experiments on adrenal medullary granules have provided evidence for the view that the decarboxylation of dopa takes place in the cell sap while the following step in the biosynthesis of noradrenaline the β hydroxylation of dopamine is dependent on the presence of granules (KIRSHNER 1959) Whether dopamine is taken up by the granules after its formation in the extragranular fluid and transformed to noradrenaline within the granules or whether the newly formed noradrenaline is formed in the cytoplasm and then taken up by the granules is not known Methylation of noradrenaline in chromaffin cells seems to take place in the cell sap (KIRSHNER and GOODALL 1957) but most of the adrenaline formed is found in the adrenal medullary granules (HILLARP 1960) Certain observations suggest that noradrenaline and adrenaline are bound to different kinds of granules which can be separated for instance by gradient centrifugation (SCHUMANN 1957 EADE 1958)

These findings and direct observations (BERTLER *et al* 1961 CARLSSON and HILLARP 1961) indicate that the adrenal medullary granules can take up catecholamines from the outside and store them Assuming similar properties for the adrenergic nerve granules it might be expected that they have the ability to take up and store the natural neurotransmitter noradrenaline The present communication deals with the uptake of exogenous noradrenaline It also seemed of interest to study the specificity of this uptake by exposing the granules also to adrenaline

Methods

As material for these studies granules obtained from bovine splenic nerves were used prepared according to a previously described method (EULER 1958) essentially consisting in squeezing the desheathed nerves between rollers at 0 °C The press juice was collected together with the washing fluid consisting of ice cold neutral isotonic potassium phosphate 5—10 ml for 1 g nerve Larger tissue particles were removed by centrifuging the slightly turbid suspension at 0—5 °C for 10 min at 600—1 000 × *g*

In most cases the fine granular suspension making up the supernatant was directly used for the incubation experiments These were carried out at temperatures of 0—37 °C in a water bath and catecholamines as hydrochlorides added as indicated in the experiments After the incubation period the suspension was centrifuged at 0—5 °C for 30 min at 50 000 × *g* which completely sedimented all catecholamine containing granules into an almost colourless, semi translucent layer

After decanting the supernatant the walls of the centrifuge tubes of plastic material were carefully wiped dry with filter paper and the sediment extracted with 1 ml 0 1 N HCl The addition of 0 1 ml 5 per cent metaphosphoric acid allowed the formation of a flocculent precipitate which when centrifuged for 10 min at 10 000 × *g* gave a clear supernatant This was used for quantitative fluorimetric assay of noradrenaline and adrenaline according to the technique of EULER and LISHAJKO (1961)

In some experiments the granular suspension was directly centrifuged the sediment resuspended in isotonic neutral K phosphate and the suspension incubated as indicated in the experiments

The net uptake of catecholamines was estimated by subtracting the amount contained in the residual incubation fluid after sedimentation from the total amount

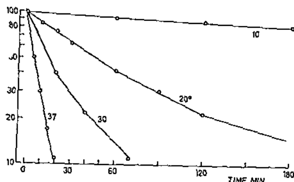


Fig 1 Isolated bovine splenic nerve granules incubated in isotonic K phosphate pH 7 0 at different temperatures Ordinate noradrenaline in sedimented granules in per cent of original amount Abscissa time in min

the sedimented granule pellet The subtractable amount was taken as the difference between the noradrenaline in the sediments obtained from suspensions with and without addition of the amine in the cold and immediately centrifuged assuming that no uptake would occur during these conditions The net uptake was also determined after sedimentation resuspension in fresh K phosphate and resedimentation The two methods gave very similar results

Spontaneous release of noradrenaline from granules

As indicated in previous communications incubation of bovine splenic nerve granules in neutral isotonic K phosphate solution causes a continuous release of noradrenaline at a rate which depends on the temperature Between 0 —5 C the release rate is low so that even after 5 hours the loss is very small This incidentally makes it possible to maintain granule suspensions for experimental purposes over many hours in the refrigerator at 0 —5 C At 20 C the release rate is much increased about 80 per cent of the total amount of noradrenaline in the granules being liberated in 2 hours following an exponential course Fig 1 also presents data from similar experiments at 10 30 and 37 C showing that the release rate increases steeply with temperature

The data show that the reaction constant increases greatly between 0 and 20 C and thereafter at a more moderate rate At 0 C the reaction rate is too slow to allow an accurate estimation of the reaction constant or Q_{10} (0 —10) The Q_{10} value is about 15 between 10 and 20 In the region 20 —37 C Q_{10} is still above 3 indicating that the reaction is not or not entirely of enzymatic character Whether the slight differences in Q_{10} for 20 —30 C and above 30 C are real or not cannot be stated with certainty

Incubation of nerve storage granules in presence of noradrenaline

While bovine splenic nerve granules when incubated at 20 C in the original suspension lose slightly over 50 per cent of their noradrenaline content

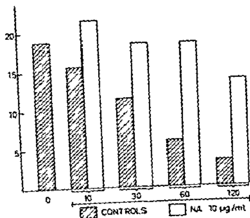


Fig 2 Conditions as in Fig 1 Striped columns show relative amount of noradrenalin (NA) in sediment of granules after incubation at 20 C for 10 30 60 and 120 min. Empty columns show relative amounts of NA in granules after incubation for the same times with NA (10 µg/ml) Ordinate NA in arbitrary units

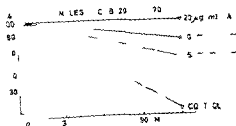


Fig 3 Conditions as in Fig 1 Content of noradrenaline (NA) in sediment after 120 min incubation at 20 C The decrease is decreased when NA (5 10 and 20 µg/ml) is present in incubation fluid Ordinate NA in sediment in per cent of original amount Abscissa time in min

in 1 hour their whole content may be retained if noradrenaline is added to the suspension This is illustrated in Fig 2 which shows the usual rate of spontaneous liberation in the controls incubated for 10—120 min at 20 C In the presence of 10 µg/ml of noradrenaline its level in the granules is maintained for a period of 60 min In this and other experiments with noradrenaline added it has been observed that there is usually some loss after 2 hours incubation using 10 µg/ml of noradrenaline As seen in Fig 3 no loss may be encountered even after 2 hours when a higher noradrenaline level is maintained (20 µg/ml) Fig 3 shows the effect of noradrenaline concentrations of 5 10 and 20 µg/ml during incubation for 2 hours at 20 C Under these conditions the sediment contains considerably higher amounts of noradrenaline after 2 hours than the control At 20 µg ml there is no difference between the original amount of noradrenaline and that found after 2 hours incubation which normally brings the noradrenaline content in the sediment down to about 20 per cent of the original With noradrenaline concentrations of 5 and 10 µg/ml in the incubation fluid the granules still retain their content considerably better than the control

If the original suspension is used as incubation fluid it must be remembered that this contains about 1 µg noradrenaline per ml representing the fr

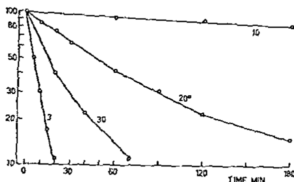


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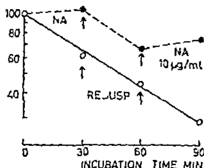


Fig 5 Full line no adrenaline rel ase curve on incubation in isotonic K phosphate pH 7 0 and 20 C. At 30 and 60 min granules were sedimented and resuspended in f esh K phosphate Broken line sam except that incubation medium con tained NA, 10 µg/ml between 0 and 0 min and betw en 60 and 90 min.

A slight net uptake was ob erved when non-depleted granules were incubated with noradrenaline 10—20 µg/ml for 2 hours at 0 C both in the original suspension and with resuspended granules

In the presence of noradrenaline in the incubation fluid the granules retain their noradrenaline less effectively at 37 C than at 20 C During incubation with 10 µg/ml noradrenaline the content of the granules is higher after 10 min at 37 C than in the control (61 per cent as compared to 31 per cent in the control) but falls thereafter at almost the same rate as the control possibly depending on inactivation of the amine After 10 min with 20 µg/ml nor adrenaline the content is relatively well maintained (85 per cent) but then falls rapidly It appears therefore that although the granules react to nor adrenaline in the ambient fluid largely in the same way at 37 C as at 20 C the ability to keep up the intragranular noradrenaline level rapidly breaks down in the system described

Spontaneous release in resuspended granules

After sedimentation and resuspension at 0 C usually a small loss of nor adrenaline from the granules of the order of 10 per cent of the original amount is observed The spontaneous release rate after resuspension usually does not differ materially from that in the original suspension over periods up to 2 hours Occasionally with resuspended granules it was observed that the release rate followed the normal course up to 1 hour but then slowed down conspicuously although the noradrenaline concentration was low in the in cubation fluid In other experiments the release rate was irregular being slow during a certain phase and then normal again The uptake of noradrenaline after partial depletion was low in these cases suggesting some functional damage to the granules

As shown above (Fig 2) the noradrenaline content in granules is unchanged after 30—60 min when incubated with noradrenaline 10 µg/ml at 20 C in the original suspension Sedimentation and resuspension of such granules 60 min incubation in isotonic K phosphate may occasionally involve a

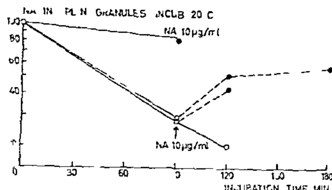


Fig 6 Uppermost line release rate in splenic nerve granules incubated 90 min in isotonic K phosphate pH 7 0 20 C in presence of noradrenaline (NA) 10 μg/ml. Empty circles release rate on incubation without NA during 90 and 120 min. Filled circles NA in sediment after 30 and 90 min further incubation in presence of NA 10 μg ml. Ordinate NA in per cent of unincubated control.

what larger loss of noradrenaline than granules which are immediately sedimented and resuspended

Fig 5 shows that granules which have maintained their noradrenaline content during incubation at 20 C with noradrenaline (10 μg/ml) after resuspension in isotonic K phosphate release their noradrenaline at the normal rate After the noradrenaline content had fallen to 68 per cent of the original in 30 min addition of noradrenaline 10 μg/ml causes the granules to retain and even gain some noradrenaline during further incubation at 20 C for 30 min

Uptake of noradrenaline in granules after depletion

In the previous described experiments no direct evidence was obtained for an actual uptake of noradrenaline at 20 C or higher temperatures since the results may be explained by an inhibition of the spontaneous loss It therefore appeared of interest to study whether an actual uptake could take place after previous depletion In these experiments the depletion was achieved by incubation for various times at 20 —30 C thus lowering the noradrenaline content of the granules to some 15—30 per cent of the original amount Noradrenaline was then added to the suspension in varying amounts and incubation continued for various lengths of time and at various temperatures

The net uptake was found to depend on the concentration of noradrenaline in the incubation fluid being marked at pH 7 0 with 20 μg/ml noradrenaline while it was considerably less with 5 and 10 μg/ml At 0 C an uptake during 30—60 min could also be demonstrated although it was smaller When the suspension was centrifuged immediately after addition of noradrenaline no definite uptake was observed

After depletion for 40—60 min at 30 C and resuspension the uptake of noradrenaline at 20 C for 60 min from the incubation fluid containing 10 —20 μg/ml was usually small suggesting that the granules after this treatment had altered some of their properties

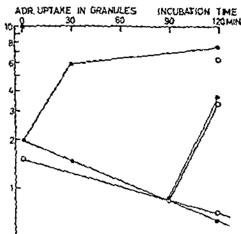


Fig 7 Upper curve adrenaline in splenic nerve granules before and after incubation in isotonic K phosphate pH 7 0 20 C for 30 and 120 min in presence of adrenaline 10 µg ml Lower curves adrenaline in granules after incubation for 30 90 and 120 min At 90 min addition of adrenaline 10 µg/ml (2 experiments) O d nat ad line in arbitrary units

Fig 6 shows the uptake after 90 min depletion at 20 C with noradrenaline 10 µg/ml at 20 C for 30 min. After that no definite further uptake was observed A considerable net uptake at 20 C during 30 min could be demonstrated even after sedimentation and resuspension following incubation with noradrenaline

Uptake of adrenaline in granules

Since the original amount of adrenaline is usually quite small in bovine splenic nerve preparations it appeared possible to study the actual uptake of adrenaline in undepleted granules provided the granules which normally store noradrenaline are also capable of taking up adrenaline In these experiments adrenaline hydrochloride was added to the incubation fluid (isotonic K phosphate pH 7 0) to a final concentration of 10 µg ml After incubation the granules were sedimented and after extraction analysed for noradrenaline and adrenaline according to EULER and LISHAJKO (1961 c)

Fig 7 shows the result of two experiments in which adrenaline was added at zero-time and after 90 min As seen in the figure there is a considerable uptake in both instances while the control figures fall indicating a gradual spontaneous loss at 20 C Continued incubation for 90 min after the first period of 30 min only slightly increases the uptake Even after incubation for 90 min without adrenaline subsequent addition of the amine causes a marked uptake The experiments clearly show that the granules are able to take up adrenaline in amounts which are similar to the uptake of noradrenaline in depleted granules

Granules which retain their noradrenaline content by addition of noradrenaline to the incubation fluid (Fig 1) take up small amounts of adrenaline only After partial depletion the granules are able to take up both amines (Fig

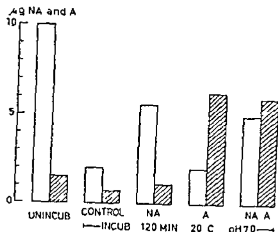


Fig. 8. Empty columns noradrenaline (NA), striped columns adrenaline (A) in splenic nerve granules. First two pairs of columns original amounts and after 120 min incubation at 20° C. Following three pairs NA and A after 120 min incubation with addition of NA, A and both amines (NA + A) each to 10 μg/ml. pH = 7.0. Ordinate NA and A in μg.

Usually only a small net increase of the sum of the amines over the original amount of noradrenaline was observed, suggesting that the amines compete for the available binding sites. In fact the total amine figures were almost identical after 30 min incubation with adrenaline alone and after 120 min incubation with adrenaline and noradrenaline. The relative amounts taken up appear to be a function of the concentrations of the amines.

In other experiments granules were partially depleted by incubation at 20° C for 90 min before addition of the amines. On incubation with these in concentrations of 10 μg/ml an uptake was observed for both amines.

Discussion

The present experiments have corroborated previous results regarding the time course of the spontaneous depletion of noradrenaline from nerve granules. The release rate increases steeply with temperature and is quite fast at 37° C in contrast to the corresponding event in bovine adrenal medullary granules (HILLARP and NILSON 1954).

The high Q_{10} values above 10 between 0 and 20° C indicate that the release process can hardly be enzymatically controlled but rather suggests a physico-chemical event of unknown kind. Even between 20 and 37° C the Q_{10} is above 3 and thus too high to be accounted for by enzyme action. As to the actual binding and release mechanisms no definite statements can be made so far. The available data point in the direction of loose bindings of some kind, possibly to phospholipids which are known to bind amines in reversible linkage. The finding that neither dinitrophenol nor ouabaine in concentrations of 0.01—100 μg/ml influence the release rate at 20° C also speaks against an active energy consuming release mechanism.

The uptake and binding is to some extent unspecific in that adrenaline may be taken up stored and released in a similar fashion to noradrenaline Non specific uptake has also been reported for adrenal medullary granules by BERTLER ROSENGREN and ROSENGREN (1960) who found that these may store small amounts of dopamine and even 5 hydroxytryptamine

The rate of uptake is markedly dependent upon the concentration of the amine in the incubation medium At 20 C a concentration of 10 μg/ml of noradrenaline seems to be the lower limit at which the content of the granules remains unchanged for a period of about 1 hour After longer periods and at higher temperatures the granules maintain their noradrenaline less efficiently This may be due to less favourable conditions of viability of the granules which apparently undergo some kind of ageing

The binding capacity of the granules is not known but it seems that the uptake is generally small in undepleted granules at pH 7 0 Thus exposure of granules to noradrenaline at 20 usually does not cause an absolute increase of their noradrenaline content but only prevents the spontaneous loss On the other hand some results indicate that the stores in the freshly prepared granules are not filled to capacity since (1) an actual uptake has been observed at 0 C (when no depletion takes place) and (2) adrenaline is taken up at a rate which in 30 min at 20 C may exceed that of the loss of noradrenaline Moreover incubation of granules in the presence of both noradrenaline and adrenaline causes in the first 30—60 min a moderate but definite increase of their total amine content of the order of 10—20 per cent of the original amount at pH 7 0

From the reported experiments it can be seen that even after extensive depletion the granules are able to take up amines to an extent approaching the normal content These results suggest that the granules possess a limited number of storage sites which may be occupied by either adrenaline or noradrenaline (and possibly other amines) Under normal conditions the storage ability of granules from bovine splenic nerves prepared with reasonable precautions particularly adequate cooling may be regarded as utilized to some 80—90 per cent judging from the supplementary uptake capacity at pH 7 0

As shown in another paper (EULER and LISHAJKO 1963) the rate of release of the amines actually taken up by depleted granules does not seem to differ from that of the undepleted granules

The significance of the ability of the granules to retain their amine content and to take up exogenous amines after depletion may be considered in connection with the physiological events of transmitter release during nerve stimulation It may be noted that the concentrations of noradrenaline allowing the granules to retain their amine content is on the same order as that presumably occurring in the axoplasm of adrenergic nerves Analysis of the amine content of bovine splenic nerves has shown that the total amount of noradrenaline is about 10—15 μg g fresh nerve (EULER 1949) Previous results

have shown that about 70 per cent of the noradrenaline occurs in free form and about 30 per cent in the granules prepared from the splenic nerves (EULER and LISHAJKO 1961 a) It might therefore be assumed that the free amine occurs in the concentration of approximately 10 μg/ml which has been found effective in preventing the loss of amine from granules which occurs at lower ambient concentrations No data are available for the concentrations of free and bound noradrenaline in the terminal parts of the adrenergic nerves There is good evidence however that the total concentration of noradrenaline is much higher than in the axons in the nerve trunk (EULER 1961 HILLARP 1959 FALCK and TORP 1962) Assuming an equilibrium between the granular bound noradrenaline and the free pool the concentration in the latter may have a similar value as in the nerve trunk although the amount relative to the bound noradrenaline in the granules may be much smaller As a working hypothesis the mechanism of transmitter release and repletion of the stores may then be envisaged in the following way At the time of the nerve impulse the permeability of the terminal axon membrane is temporarily increased allowing some of the free amine present in the axon to diffuse out and reach the receptors The ensuing lowering of the amine concentration in the cytoplasm of the nerve terminal creates a condition during which the granules are no longer able to retain their contents which are given off to the surrounding axoplasm thus repleting the immediately accessible pool of transmitter The partial depletion of the amine content of the granules on the other hand leaves storage sites available for uptake Assuming that the release of nor adrenaline from the granules triggers biosynthesis by some as yet unknown mechanism the stores of the granules are refilled and the newly synthesized amine partially transferred to the free pool maintaining an equilibrium between free and bound amine Likewise it may be assumed that biosynthesis comes to a stop when the granule stores are completely filled and the con centration of the free pool has become high enough to prevent further release from the granules (cf SCHARRER and BROWN 1961)

The rapid release and uptake of amines in isolated granules at 37 C makes them eminently suitable as stores for the amine The possibility might be considered that biosynthesis is regulated automatically by the equilibrium between the stores in the granules and the free pool thereby offering a supply for immediate use The rapidity of the replenishing process is borne out by the fact that the noradrenaline content of an organ is nearly maximal even after prolonged stimulation of the adrenergic nerves (EULER and HELLNER BJORKMAN 1955) and that exhaustion of the transmitter does not occur under normal conditions (LUCO and GONI 1948)

The demonstration of an uptake also of adrenaline calls for some comment It might be assumed from this fact that storage granules are able to store adrenaline if this amine is offered in a sufficiently high concentration There are good reasons to believe that this would actually be the case if adrenaline

and not noradrenaline were produced by the biosynthetic process There is no support for this assumption however The concentration of adrenaline in the blood coming from other sources in the body would hardly reach concen trations anywhere near those necessary in order to compete effectively with noradrenaline for the granules and the free pool An adrenaline uptake might occur however under certain experimental conditions for instance in a *depleted transmitter system if adrenaline is injected intravenously in large* quantities In fact some observations seem to support such an assumption Thus BURN and RAND (1960) and ROSELL and SEDVALL (1961) have observed in reserpinized cats that stimulation of adrenergic nerves produces a temporary effect after injections of adrenaline as well as noradrenaline This indicates an uptake and storage of either amine presumably both in the free pool and *secondarily in the granules*

This is also in agreement with the work of HERTTING and AXELROD (1961) who found that tritiated noradrenaline is taken up by organs and released upon stimulation An uptake was not found in organs after adrenergic nerve de generation indicating that the uptake is in the nerve axons

The uptake of injected noradrenaline in dog hearts has been studied by *WEGMANN and KAKO (1961) They found that about 7o per cent of the cate* cholamines normally present in the homogenate were located in the high speed centrifugation sediment After injection of noradrenaline 10 mg/kg intra peritoneally most of the catecholamines were found in the supernatant No statistically significant difference was found between the noradrenaline con tent in the control sediment and after noradrenaline injection indicating that the uptake *in granules was at most ery slight* Even a minimal uptake would be demonstrable with radioactively labelled catecholamines however From a quantitative point of view such an uptake would probably be of small significance normally

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Intercostal γ-Motor Activity

By

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The activity of γ motor fibres has been directly recorded together with α motor fibres from fine branches of the muscle nerve to the external intercostal muscle in cats under light nembutal anaesthesia The assignation of a spike to either the α or the γ fibre group has been based on the method of selective blocking (MATTHEWS and RUSHWORTH 1957) using 0 125 % lidocaine solution (B and D of Fig 1) When only one or two γ fibres were present in the nerve twig the spikes were identified by determination of their conduction velocity from a stimulus site on the ventral root

During spontaneous breathing the activity of the γ fibres was either rhythmic with respiration (Fig 1 A) or steady (Fig 1 C) The rhythmic γ discharge appeared synchronously with the α activity (CRITCHLOW and EULER 1962) The γ discharge of both types could be modified from spinal and supraspinal levels Both excitatory and inhibitory effects were observed The γ activity was more readily influenced from the skin than the α activity (c f Fig 1 E) Hypercapnia hypoxia and asphyxia increases the discharge in some γ fibres whereas hyperventilation decreases this activity These effects are absent after high spinalization

Complete curarization did not abolish the γ discharge that occurred in pace with the animal's own rhythm indicating that these γ motoneurones are rhythmically activated from medullary structures During artificial hyperventilation and after high spinalization however rhythmic γ discharge often appeared synchronously with the respiratory pump in phase either with the inflation as in F of the Fig or the deflation This indicates that rhythmic γ activity may be driven by spinal reflexes (CRITCHLOW and EULER 1962)

A B 1sec 30ml C D E 1sec 30ml 500cps F 1sec 5mmHg

Fig 1 Efferent activity from three different nerve twigs to external intercostal muscle A Spontaneous breathing one α- and one γ-fibre B The latter selectively blocked by lidocaine C Spontaneous breathing three α- and one γ-fibres γ-conduction velocity 25 m/sec D The latter selectively blocked by lidocaine E Single shocks to the lateral cutaneous branch of the intercostal nerve of the same segment 5 superimposed sweep Reflex response only from the γ-fibre F Artificial respiration Spinal cord cut at C Only one γ-fibre conduction velocity 20 m sec Note firing in phase with inflation Lower tracings A—D tidal volume upward deflection inspiration F intratracheal pressure upward deflection inflation

Mechanical stimulation applied to the intercostal muscles of adjacent segments caused excitatory or inhibitory changes in the γ discharge Electrical stimulation of the ventral root of an adjacent segment activated the γ motoneurones Further succinylcholine administered intravenously in doses of 0 5—1 mg/kg bodywt caused reflex activation not only of the α motoneurones (HENATSCH 1962) but also of the γ motoneurones These results suggest that inspiratory γ motoneurones may be proprioceptively excited

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